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(57) Abstract

This invention relates to newly identified pancreas or pancreatic cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "pancreatic cancer antigens", and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such pancreatic cancer antigens for detection, prevention and treatment of disorders of the pancreas, particularly the presence of pancreatic cancer. This invention relates to the pancreatic cancer antigens as well as vectors, host cells, antibodies directed to pancreatic cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

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Human Pancreas and Pancreatic Cancer Associated Gene Sequences and Polypeptides

Field of the Invention

This invention relates to newly identified pancreas or pancreatic cancer related polynucleotides and the polypeptides encoded by these polynucleotides herein collectively known as "pancreatic cancer antigens," and to the complete gene sequences associated therewith and to the expression products thereof, as well as the use of such pancreatic cancer antigens for detection, prevention and treatment of disorders of the pancreas, particularly the presence of pancreatic cancer. This invention relates to the pancreatic cancer antigens as well as vectors, host cells, antibodies directed to pancreatic cancer antigens and recombinant and synthetic methods for producing the same. Also provided are diagnostic methods for diagnosing and treating, preventing and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention. The present invention further relates to methods and/or compositions for inhibiting the production and/or function of the polypeptides of the present invention.

Background of the Invention

Cell growth is a carefully regulated process which responds to specific needs of the body. Occassionally, the intricate, and highly regulated controls dictating the rules for cellular division break down. When this occurs, the cell begins to grow and divide independently of its homeostatic regulation resulting in a condition commonly referred to as cancer. In fact, cancer is the second leading cause of death among Americans aged 25-44.

Pancreatic cancer is one of the most dangerous cancers, killing half its victims within 6 weeks and having a 5-year survival rate of only 1%. The diagnosis of pancreatic carcinoma is often associated with a poor prognosis, because most patients already have advanced disease. Despite the many advances reported during the past few years, pancreatic cancer remains a profound therapeutic challenge. It is hoped that the increasing knowledge of the

molecular biology of pancreatic carcinoma will lead to improvements in diagnosing, staging, and treating pancreatic adenocarcinoma (Brand et al., Curr Opin Oncol 10:362-6 (1998)).

There is a need, therefore, for identification and characterization of factors that modulate activation and differentiation of pancreatic cells, both normally and in disease states. In particular, there is a need to isolate and characterize additional molecules that mediate apoptosis, DNA repair, tumor-mediated angiogenesis, genetic imprinting, immune responses to tumors and tumor antigens and, among other things, that can play a role in detecting, preventing, ameliorating or correcting dysfunctions or diseases related to the pancreas.

Summary of the Invention

The present invention includes isolated nucleic acid molecules comprising, or alternatively, consisting of, a pancreas and/or pancreatic cancer associated polynucleotide sequence disclosed in the sequence listing (as SEQ ID NOs:1 to 459) and/or contained in a human cDNA clone described in Tables 1, 2 and 5 and deposited with the American Type Culture Collection ("ATCC"). Fragments, variant, and derivatives of these nucleic acid molecules are also encompassed by the invention. The present invention also includes isolated nucleic acid molecules comprising, or alternatively consisting of, a polynucleotide encoding a pancreas and/or pancreatic cancer polypeptide. The present invention further includes pancreas and/or pancreatic cancer polypeptides encoded by these polynucleotides. Further provided for are amino acid sequences comprising, or alternatively consisting of, pancreas and/or pancreatic cancer polypeptides as disclosed in the sequence listing (as SEQ ID NOs: 460 to 918) and/or encoded by a human cDNA clone described in Tables 1, 2 and 5 and deposited with the ATCC. Antibodies that bind these polypeptides are also encompassed by the invention. Polypeptide fragments, variants, and derivatives of these amino acid sequences are also encompassed by the invention, as are polynucleotides encoding these polypeptides and antibodies that bind these polypeptides. Also provided are diagnostic methods for diagnosing and treating, preventing, and/or prognosing disorders related to the pancreas, including pancreatic cancer, and therapeutic methods for treating such disorders. The invention further relates to screening methods for identifying agonists and antagonists of pancreatic cancer antigens of the invention.

Detailed Description

Tables

Table 1 summarizes some of the pancreatic cancer antigens encompassed by the invention (including contig sequences (SEQ ID NO:X) and the cDNA clone related to the contig sequence) and further summarizes certain characteristics of the pancreatic cancer polynucleotides and the polypeptides encoded thereby. The first column shows the "SEQ ID NO:" for each of the 459 pancreatic cancer antigen polynucleotide sequences of the invention. The second column provides a unique "Sequence/Contig ID" identification for each pancreas and/or pancreatic cancer associated sequence. The third column, "Gene Name," and the fourth column, "Overlap," provide a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database and the database accession no. for the database sequence having similarity, respectively. The fifth and sixth columns provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity), respectively, observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence. The ninth column provides a unique "Clone ID" for a cDNA clone related to each contig sequence.

Table 2 summarizes ATCC Deposits, Deposit dates, and ATCC designation numbers of deposits made with the ATCC in connection with the present application.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, fifteen or more of any one or more of these public EST sequences are optionally excluded from certain embodiments of the invention.

Table 4 lists residues comprising antigenic epitopes of antigenic epitope-bearing fragments present in most of the pancreas and/or pancreatic cancer associated polynucleotides described in Table 1 as predicted by the inventors using the algorithm of Jameson and Wolf, (1988) Comp. Appl. Biosci. 4:181-186. The Jameson-Wolf antigenic analysis was performed using the computer program PROTEAN (Version 3.11 for the Power MacIntosh, DNASTAR, Inc., 1228 South Park Street Madison, WI). Pancreas and pancreatic cancer associated polypeptides (e.g., SEQ ID NO:Y, polypeptides encoded by SEQ ID NO:X,

or polypeptides encoded by the cDNA in the referenced cDNA clone) may possess one or more antigenic epitopes comprising residues described in Table 4. It will be appreciated that depending on the analytical criteria used to predict antigenic determinants, the exact address of the determinant may vary slightly. The residues and locations shown in column two of Table 4 correspond to the amino acid sequences for most pancreas and/or pancreatic cancer associated polypeptide sequence shown in the Sequence Listing.

Table 5 shows the cDNA libraries sequenced, and ATCC designation numbers and vector information relating to these cDNA libraries.

Definitions

The following definitions are provided to facilitate understanding of certain terms used throughout this specification.

In the present invention, "isolated" refers to material removed from its original environment (e.g., the natural environment if it is naturally occurring), and thus is altered "by the hand of man" from its natural state. For example, an isolated polynucleotide could be part of a vector or a composition of matter, or could be contained within a cell, and still be "isolated" because that vector, composition of matter, or particular cell is not the original environment of the polynucleotide. The term "isolated" does not refer to genomic or cDNA libraries, whole cell total or mRNA preparations, genomic DNA preparations (including those separated by electrophoresis and transferred onto blots), sheared whole cell genomic DNA preparations or other compositions where the art demonstrates no distinguishing features of the polynucleotide/sequences of the present invention.

As used herein, a "polynucleotide" refers to a molecule having a nucleic acid sequence contained in SEQ ID NO:X (as described in column 1 of Table 1) or the related cDNA clone (as described in column 9 of Table 1 and contained within a library deposited with the ATCC). For example, the polynucleotide can contain the nucleotide sequence of the full length cDNA sequence, including the 5' and 3' untranslated sequences, the coding region, as well as fragments, epitopes, domains, and variants of the nucleic acid sequence. Moreover, as used herein, a "polypeptide" refers to a molecule having an amino acid sequence encoded by a polynucleotide of the invention as broadly defined (obviously

excluding poly-Phenylalanine or poly-Lysine peptide sequences which result from translation of a polyA tail of a sequence corresponding to a cDNA).

In the present invention, "SEQ ID NO:X" was often generated by overlapping sequences contained in multiple clones (contig analysis). A representative clone containing all or most of the sequence for SEQ ID NO:X is deposited at Human Genome Sciences, Inc. (HGS) in a catalogued and archived library. As shown in column 9 of Table 1, each clone is identified by a cDNA Clone ID. Each Clone ID is unique to an individual clone and the Clone ID is all the information needed to retrieve a given clone from the HGS library. In addition to the individual cDNA clone deposits, most of the cDNA libraries from which the clones were derived were deposited at the American Type Culture Collection (hereinafter "ATCC"). Table 5 provides a list of the deposited cDNA libraries. One can use the Clone ID to determine the library source by reference to Tables 2 and 5. Table 5 lists the deposited cDNA libraries by name and links each library to an ATCC Deposit. Library names contain four characters, for example, "HTWE." The name of a cDNA clone ("Clone ID") isolated from that library begins with the same four characters, for example "HTWEP07". As mentioned below, Table 1 correlates the Clone ID names with SEQ ID NOs. Thus, starting with a SEQ ID NO, one can use Tables 1, 2 and 5 to determine the corresponding Clone ID, from which library it came and in which ATCC deposit the library is contained. Furthermore, it is possible to retrieve a given cDNA clone from the source library by techniques known in the art and described elsewhere herein. The ATCC is located at 10801 University Boulevard, Manassas, Virginia 20110-2209, USA. The ATCC deposits were made persuant to the terms of the Budapest Treaty on the international recognition of the deposit of microorganisms for the purposes of patent procedure.

A "polynucleotide" of the present invention also includes those polynucleotides capable of hybridizing, under stringent hybridization conditions, to sequences contained in SEQ ID NO:X, or the complement thereof (e.g., the complement of any one, two, three, four, or more of the polynucleotide fragments described herein), and/or sequences contained in the related cDNA clone within a library deposited with the ATCC. "Stringent hybridization conditions" refers to an overnight incubation at 42 degree C in a solution comprising 50% formamide, 5x SSC (750 mM NaCl, 75 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μg/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1x SSC at about 65 degree C.

Also included within "polynucleotides" of the present invention are nucleic acid molecules that hybridize to the polynucleotides of the present invention at lower stringency hybridization conditions. Changes in the stringency of hybridization and signal detection are primarily accomplished through the manipulation of formamide concentration (lower percentages of formamide result in lowered stringency); salt conditions, or temperature. For example, lower stringency conditions include an overnight incubation at 37 degree C in a solution comprising 6X SSPE (20X SSPE = 3M NaCl; 0.2M NaH_2PO_4; 0.02M EDTA, pH 7.4), 0.5% SDS, 30% formamide, 100 ug/ml salmon sperm blocking DNA; followed by washes at 50 degree C with 1XSSPE, 0.1% SDS. In addition, to achieve even lower stringency, washes performed following stringent hybridization can be done at higher salt concentrations (e.g. 5X SSC).

Note that variations in the above conditions may be accomplished through the inclusion and/or substitution of alternate blocking reagents used to suppress background in hybridization experiments. Typical blocking reagents include Denhardt's reagent, BLOTTO, heparin, denatured salmon sperm DNA, and commercially available proprietary formulations. The inclusion of specific blocking reagents may require modification of the hybridization conditions described above, due to problems with compatibility.

Of course, a polynucleotide which hybridizes only to polyA+ sequences (such as any 3' terminal polyA+ tract of a cDNA shown in the sequence listing), or to a complementary stretch of T (or U) residues, would not be included in the definition of "polynucleotide," since such a polynucleotide would hybridize to any nucleic acid molecule containing a poly (A) stretch or the complement thereof (e.g., practically any double-stranded cDNA clone generated using oligo dT as a primer).

The polynucleotides of the present invention can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, polynucleotides can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, the polynucleotide can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide may also contain one or more modified bases or DNA or

RNA backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

In specific embodiments, the polynucleotides of the invention are at least 15, at least 30, at least 50, at least 100, at least 125, at least 500, or at least 1000 continuous nucleotides but are less than or equal to 300 kb, 200 kb, 100 kb, 50 kb, 15 kb, 10 kb, 7.5kb, 5 kb, 2.5 kb, 2.0 kb, or 1 kb, in length. In a further embodiment, polynucleotides of the invention comprise a portion of the coding sequences, as disclosed herein, but do not comprise all or a portion of any intron. In another embodiment, the polynucleotides comprising coding sequences do not contain coding sequences of a genomic flanking gene (i.e., 5' or 3' to the gene of interest in the genome). In other embodiments, the polynucleotides of the invention do not contain the coding sequence of more than 1000, 500, 250, 100, 50, 25, 20, 15, 10, 5, 4, 3, 2, or 1 genomic flanking gene(s).

"SEQ ID NO:X" refers to a pancreatic cancer antigen polynucleotide sequence described in Table 1. SEQ ID NO:X is identified by an integer specified in column 1 of Table 1. The polypeptide sequence SEQ ID NO:Y is a translated open reading frame (ORF) encoded by polynucleotide SEQ ID NO:X. There are 459 pancreatic cancer antigen polynucleotide sequences described in Table 1 and shown in the sequence listing (SEQ ID NO:1 through SEQ ID NO:459). Likewise there are 459 polypeptide sequences shown in the sequence listing, one polypeptide sequence for each of the polynucleotide sequences (SEQ ID NO:460 through SEQ ID NO:918). The polynucleotide sequences are shown in the sequence listing immediately followed by all of the polypeptide sequences. Thus, a polypeptide sequence corresponding to polynucleotide sequence SEQ ID NO:1 is the first polypeptide sequence shown in the sequence listing. The second polypeptide sequence corresponds to the polynucleotide sequence shown as SEQ ID NO:2, and so on. In otherwords, since there are 459 polynucleotide sequences, for any polynucleotide sequence SEQ ID NO:X, a corresponding polypeptide SEQ ID NO:Y can be determined by the formula X + 459 = Y. In addition, any of the unique "Sequence/Contig ID" defined in column 2 of Table 1, can be linked to the corresponding polypeptide SEQ ID NO:Y by reference to Table 4.

The polypeptides of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may

contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann NY Acad Sci 663:48-62 (1992).)

The pancreas and pancreatic cancer polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Means for preparing such polypeptides are well understood in the art.

The polypeptides may be in the form of the secreted protein, including the mature form, or may be a part of a larger protein, such as a fusion protein (see below). It is often

advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification, such as multiple histidine residues, or an additional sequence for stability during recombinant production.

The pancreas and pancreatic cancer polypeptides of the present invention are preferably provided in an isolated form, and preferably are substantially purified. A recombinantly produced version of a polypeptide, including the secreted polypeptide, can be substantially purified using techniques described herein or otherwise known in the art, such as, for example, by the one-step method described in Smith and Johnson, Gene 67:31-40 (1988). Polypeptides of the invention also can be purified from natural, synthetic or recombinant sources using techniques described herein or otherwise known in the art, such as, for example, antibodies of the invention raised against the polypeptides of the present invention in methods which are well known in the art.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

"A polypeptide having functional activity" refers to polypeptides exhibiting activity similar, but not necessarily identical to, an activity of a polypeptide of the present invention, including mature forms, as measured in a particular assay, such as, for example, a biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

The functional activity of the pancreatic cancer antigen polypeptides, and fragments, variants derivatives, and analogs thereof, can be assayed by various methods.

For example, in one embodiment where one is assaying for the ability to bind or compete with full-length polypeptide of the present invention for binding to an antibody to

the full length polypeptide antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays, hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In another embodiment, where a ligand is identified, or the ability of a polypeptide fragment, variant or derivative of the invention to multimerize is being evaluated, binding can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky, E., et al., Microbiol. Rev. 59:94-123 (1995). In another embodiment, physiological correlates polypeptide of the present invention binding to its substrates (signal transduction) can be assayed.

In addition, assays described herein (see Examples) and otherwise known in the art may routinely be applied to measure the ability of polypeptides of the present invention and fragments, variants derivatives and analogs thereof to elicit polypeptide related biological activity (either in vitro or in vivo). Other methods will be known to the skilled artisan and are within the scope of the invention.

Pancreas and Pancreatic Cancer Associated Polynucleotides and Polypeptides of the Invention

It has been discovered herein that the polynucleotides described in Table 1 are expressed at significantly enhanced levels in human pancreas and/or pancreatic cancer tissues. Accordingly, such polynucleotides, polypeptides encoded by such polynucleotides,

and antibodies specific for such polypeptides find use in the prediction, diagnosis, prevention and treatment of pancreas related disorders, including pancreatic cancer as more fully described below.

Table 1 summarizes some of the polynucleotides encompassed by the invention (including contig sequences (SEQ ID NO:X) and the related cDNA clones) and further summarizes certain characteristics of these pancreas and/or pancreatic cancer associated polynucleotides and the polypeptides encoded thereby.

Table 1

Seq ID No.	Sequence/ Contig ID	Gene Name	Overlap	HGS Nucleotide Start	HGS Nucleotide End	% Identity	% Similarity	Clone ID
1	456379			3	197			HCDAJ28
2	462108	5'-AMP-activated protein kinase, gamma-1 subunit [Homo sapiens] >sp|P54619|AAKG_HUMAN 5'-AMP-ACTIVATED PROTEIN KINASE, GAMMA-1 SUBUNIT (AMPK GAMMA-1 CHAIN). Length = 331	gi|1335856	2	1033	100	100	HHPDJ86
3	503446			161	415			HTTEU40
4	507841			1	159			HOAAF52
5	509287	Similarity to Yeast SOH-1 protein (SW:P38633) [Caenorhabditis elegans] >sp|P91869|P91869 F32H2.2 PROTEIN. Length = 163	gnl|PID|e1346272	1	480	66	80	HMTME61
6	509672			16	141			HTPAM31
7	509673			1	162			HMWBR61
8	518767			2	148			HTPBZ88
9	522008			1	378			HDPFM59
10	524112			248	475			HJPCM86
11	525971	clk2-136; putative [Homo sapiens] >pir|S53638|S53638 protein kinase clk2-139 (EC 2.7.1.-) - human Length = 139	gi|632966	1	579	95	97	HTXAR16

12	527156	ORF YOR262w [Saccharomyces cerevisiae] >pir|S67159|S67159 probable membrane protein YOR262w - yeast (Saccharomyces cerevisiae) >sp|Q08726|Q08726 CHROMOSOME XV READING FRAME ORF YOR262W. Length = 347	gnl|PID|e252113	174	506	48	63	HMWFW50
13	532502			73	267			HTPCP39
14	533459	electron transfer flavoprotein beta subunit [Homo sapiens] >pir|S32482|S32482 electron transfer flavoprotein beta chain - human >sp|P38117|ETFB_HUMAN ELECTRON TRANSFER FLAVOPROTEIN BETA-SUBUNIT (BETA-ETF). Length = 255	gi|297902	2	850	87	87	HKAAY56
15	533551	interleukin 4 receptor [Homo sapiens] >gi|3219334 (AC004525) Interleukin 4 alpha-chain precursor [Homo sapiens] >sp|P24394|IL4R_HUMAN INTERLEUKIN-4 RECEPTOR ALPHA CHAIN PRECURSOR (IL-4R-ALPHA) (CD124 ANTIGEN). Length = 825	gi|33834	2	1564	84	84	HTOEV80
16	537850	tetraspan membrane protein [Homo sapiens] >sp|P48230|IL T4_HUMAN TETRASPAN MEMBRANE PROTEIN IL-TMP. Length = 202	gi|953239	179	913	89	89	HLDQN04
17	537925	sialyltransferase [Homo sapiens] >pir|A54898|A54898 gal-beta1,3galNAc alpha-2,3-sialyltransferase (EC 2.4.99.-) - human Length = 340	gi|522197	1	228	80	82	HSKYV64

18	538160	pancreatic zymogen granule membrane protein GP-2 [Homo sapiens] >pir|G02091|G02091 pancreatic zymogen granule membrane protein GP-2 - human >sp|P55259|GP2_HUMAN PANCREATIC SECRETORY GRANULE MEMBRANE MAJOR GLYCOPROTEIN GP2 PRECURSOR (PANCREATIC ZYMOGEN GRA	gi|1244512	68	595	98	98	HPWAR18
19	540420	Method: conceptual translation supplied by author; putative hybrid protein similar to HERV-H protease and HERV-E integrase [Human endogenous retrovirus] >sp|Q68997|Q68997 SIMILAR TO HERV-H PROTEASE AND HERV-E INTEGRASE. >gi|2587023 (AF026246) HERV-E integ	gi|1049231	48	338	68	77	HTPBW19
20	540802	annexin III [Homo sapiens] >gi|178697 1,2-cyclic-inositol-phosphate phosphodiesterase [Homo sapiens] >gi|307115 lipocortin-III [Homo sapiens] >pir|A47658|LUHU3 annexin III - human >sp|P12429|ANX3_HUMAN ANNEXIN III (LIPOCORTIN III) (PLACENTAL ANTICOAGULANT	gi|410202	198	671	99	99	HMEKG44
21	540989	lipase related protein 1 [Homo sapiens] >pir|A43357|A43357 pancreatic lipase-related protein 1 - human >sp|P54315|LIP1_HUMAN PANCREATIC LIPASE RELATED PROTEIN 1 PRECURSOR (EC 3.1.1.3). Length = 467	gi|187230	29	343	98	98	HPASD60

22	540997	lipase related protein 1 [Homo sapiens] >pir|A43357|A43357 pancreatic lipase-related protein 1 - human >sp|P54315|LIP1_HUMAN PANCREATIC LIPASE RELATED PROTEIN 1 PRECURSOR (EC 3.1.1.3). Length = 467	gi|187230	2	409	98	99	HPASG94
23	548735	P69 2-5A synthase II - human Length = 727	pir|B42665|B42665	101	664	96	98	HMTAE33
24	549709	cytochrome P450 PCN3 [Homo sapiens] >pir|A34101|A34101 cytochrome P450 3A5 - human >sp|P20815|CP35_HUMAN CYTOCHROME P450 3A5 (EC 1.14.14.1) (CYPIIIA5) (P450-PCN3). >gi|950342 cytochrome P450 [Homo sapiens] {SUB 1-24} Length = 502	gi|181346	72	305	64	64	HSBZ89
25	550007	thimet oligopeptidase [Homo sapiens] >gi|1030055 metalloproteinase [Homo sapiens] >pir|JC4197|HYHUTH thimet oligopeptidase (EC 3.4.24.15) - human >sp|P52888|MEPD_HUMAN THIMET OLIGOPEPTIDASE (EC 3.4.24.15) (ENDOPEPTIDASE 24.15) (MP78). {SUB 2-689} Length =	gi|1098600	150	782	100	100	HCEIB78

26	550118	macrophage capping protein [Homo sapiens] >pir|A43358|A43358 macrophage capping protein - human >sp|P40121|CAPG_HUMAN MACROPHAGE CAPPING PROTEIN (ACTIN-REGULATORY PROTEIN CAP-G). >gi|515505 Cap-G [Homo sapiens] {SUB 1-172} Length = 348	gi|187456	1	1113	99	99	HDPXJ18
27	550148			274	456			HSBA17
28	550870	carbonic anhydrase IV [Homo sapiens] >pir|A45745|CRHU4 carbonate dehydratase (EC 4.2.1.1) IV precursor - human >sp|P22748|CAH4_HUMAN CARBONIC ANHYDRASE IV PRECURSOR (EC 4.2.1.1) (CARBONATE DEHYDRATASE IV). Length = 312	gi|179791	1	594	94	94	HNFIA35
29	552506	preproglucagon [Homo sapiens] >pir|A24377|GCHU glucagon precursor - human >sp|P01275|GLUC_HUMAN GLUCAGON PRECURSOR. >gi|31778 Human gene encoding preproglucagon. Glucagon is a 29-amino acid pancreatic hormone which counteracts the blood glucose-lowering a	gi|183270	3	350	96	96	HTPDP78

30	553765	complement factor B [Homo sapiens] >pir|S34075|BBHU complement factor B precursor - human >sp|P00751|CFAB_HUMAN COMPLEMENT FACTOR B PRECURSOR (EC 3.4.21.47) (C3/C5 CONVERTASE) (PROPERDIN FACTOR B) (GLYCINE-RICH BETA GLYCOPROTEIN) (GBG) (PBF2). >gi|758090	gi|297569	48	1214	100	100	HLDRK20
31	554050	histidyl-tRNA synthetase [Homo sapiens] >pir|I37559|SYHUHT histidine--tRNA ligase (EC 6.1.1.21) - human >gi|431312 histidyl tRNA synthetase [Homo sapiens] {SUB 1-30} Length = 509	gi|32460	374	934	86	86	HARME85
32	554186	brain glycogen phosphorylase [Homo sapiens] >pir|A29949|A29949 glycogen phosphorylase (EC 2.4.1.1), brain (astrocytoma cell line) - human Length = 863	gi|307200	2	814	98	99	HKACY69
33	554716	transcobalamin I precursor [Homo sapiens] >pir|A34227|A34227 transcobalamin I precursor - human Length = 433	gi|307479	1	441	97	97	HCHAC67
34	556791	integrin alpha 1 subunit - human (fragment) >sp|P56199|ITA1_HUMAN INTEGRIN ALPHA-1 (LAMININ AND COLLAGEN RECEPTOR) (VLA-1) (CD49A). Length = 1151	pir|A45226|A45226	2	484	93	93	HFIYR48

<table>
<tr><td>35</td><td>557121</td><td>gamma-glutmyl transpeptidase-related protein [Homo sapiens] >pir|A41125|A41125 gamma-glutamyltransferase (EC 2.3.2.2) related protein - human >sp|P36269|GGT5_HUMAN GAMMA-GLUTAMYLTRANSPEPTIDASE 5 PRECURSOR (EC 2.3.2.2) (GAMMA-GLUTAMYLTRANSFERASE 5) (GGT-R</td><td>gi|183142</td><td>151</td><td>567</td><td>100</td><td>100</td><td>HISCL81</td></tr>
<tr><td>36</td><td>557199</td><td>180 kDa bullous pemphigoid antigen 2/type XVII collagen [Homo sapiens] >sp|E307563|E307563 180 KDA BULLOUS PEMPHIGOID ANTIGEN 2/TYPE XVII COLLAGEN. >sp|G1877435|G1877435 180 KDA BULLOUS PEMPHIGOID ANTIGEN 2/TYPE XVII COLLAGEN. Length = 1497</td><td>gi|1877435</td><td>2</td><td>646</td><td>81</td><td>81</td><td>HPDDA57</td></tr>
<tr><td>37</td><td>557293</td><td>alpha-5 type IV collagen [Homo sapiens] >gi|180825 collagen type IV alpha 5 chain [Homo sapiens] {SUB 833-1604} Length = 1604</td><td>gi|1314210</td><td>3</td><td>929</td><td>99</td><td>99</td><td>HISBT90</td></tr>
<tr><td>38</td><td>557441</td><td>neurofibromin [Rattus norvegicus] >pir|JC5196|JC5196 neurofibromin I - rat >sp|P97526|P97526 NEUROFIBROMIN. >gi|309451 neurofibromin [Mus musculus] {SUB 1-96} >gi|1084091 neurofibromatosis 1 [Homo sapiens] {SUB 97-161} >gi|1084092 neurofibromatosis 1 [Hom</td><td>gnl|PID|d1008732</td><td>207</td><td>326</td><td>100</td><td>100</td><td>HTPAD51</td></tr>
</table>

39	558091	flavin-containing monooxygenase 5 [Homo sapiens] >pir|S71618|S71618 dimethylaniline monooxygenase (N-oxide-forming) (EC 1.14.13.8) FMO5 - human >sp|P49326|FMO5_HUMAN DIMETHYLANILINE MONOOXYGENASE [N-OXIDE FORMING] 5 (EC 1.14.13.8) (HEPATIC FLAVIN-CONTAINI	gi|559046	803	1066	98	98	HE9GG94
40	558423	translin [Homo sapiens] >pir|S51738|S51738 translin - human >sp|Q15631|Q15631 TRANSLIN. >gnl|PID|e313773 MTRANCDS [Homo sapiens] {SUB 23-215} Length = 228	gi|607130	49	807	93	93	HDTDF09
41	558465	G/T mismatch-specific thymine DNA glycosylase [Homo sapiens] Length = 410	gi|1378107	1	507	90	91	HAMFJ55
42	558493	tubulin beta-1 chain - slime mold (Physarum polycephalum) (fragment) >gi|313801 beta-tubulin [Physarum polycephalum] {SUB 1-203} Length = 204	pir|S02532|S02532	1	357	76	86	HTTEJ40
43	558778	T-cell antigen receptor (AA 1 - 292) [Homo sapiens] >pir|S03421|S03421 T-cell receptor delta chain precursor (Peer) - human Length = 292	gi|37004	290	625	95	95	HISBP61
44	558818	tRNA-Guanine Transglycosylase [Homo sapiens] >pir|G01932|G01932 tRNA-Guanine Transglycosylase - human Length = 494	gi|940182	1	468	100	100	HPIBT63

45	563182	(AF072128) claudin-2 [Mus musculus] >sp|O88552|O88552 CLAUDIN-2. Length = 230	gi|3335184	2	466	81	85	HCHMQ60
46	572571			194	553			HAICW02
47	575525	Bat2 [Homo sapiens] >pir|S37671|S37671 bat2 protein - human Length = 1870	gi|29375	553	858	85	87	HAPOJ89
48	580659			695	1003			HBICR03
49	583650	islet regenerating protein [Homo sapiens] >pir|A35197|RGHU1A regenerating islet lectin 1-alpha precursor - human >sp|P05451|LITA_HUMAN LITHOSTATHINE 1 ALPHA PRECURSOR (PANCREATIC STONE PROTEIN) (PSP) (PANCREATIC THREAD PROTEIN) (PTP) (ISLET OF LANGERHANS	gi|190979	261	413	78	83	HTPDS26
50	584698			982	1200			HLQCJ79
51	585791	B61 [Homo sapiens] >pir|A36377|A36377 B61 protein precursor - human >sp|P20827|EFA1_HUMAN EPHRIN-A1 PRECURSOR (EPH-RELATED RECEPTOR TYROSINE KINASE LIGAND 1) (LERK-1) (IMMEDIATE EARLY RESPONSE PROTEIN B61) (TUMOR NECROSIS FACTOR, ALPHA-INDUCED PROTEIN 4).	gi|179321	52	705	95	95	HSIDT08

52	587229	phospholipase [Homo sapiens] >gi|387025 phospholipase [Homo sapiens] >gi|2769697 (AC003982) Phosphatidylcholine 2-acylhydrolase [Homo sapiens] >pir|C25793|PSHU phospholipase A2 (EC 3.1.1.4) precursor, pancreatic - human >sp|P04054|PA21_HUMAN PHOSPHOLIPASE	gi|190013	3	470	81	81	HPDDF22
53	587246	probable transmembrane protein TMC - human Length = 705	pir|S70029|S70029	67	573	92	92	HLWAE93
54	587486	alpha-subunit of prolyl 4-hydroxylase [Homo sapiens] >pir|I37173|DAHUA2 procollagen-proline dioxygenase (EC 1.14.11.2) alpha chain precursor, splice form 2 - human >sp|P13674|P4HA_HUMAN PROLYL 4-HYDROXYLASE ALPHA SUBUNIT PRECURSOR (EC 1.14.11.2). Length =	gi|602675	745	1734	95	95	HDPWQ32
55	589218			6	185			HBSAL59
56	592154	adenylyl cyclase [Homo sapiens] >gi|395275 adenylate cyclase [Homo sapiens] >pir|I37136|I37136 adenylate cyclase (EC 4.6.1.1) - human (fragment) >sp|Q08462|CYA2_HUMAN ADENYLATE CYCLASE, TYPE II (EC 4.6.1.1) (ATP PYROPHOSPHATE-LYASE) (ADENYLYL CYCLASE) (FR	gi|763444	101	1033	95	95	HAOAE42

57	598664	unnamed protein product [unidentified] >gi|35330 carboxypeptidase a [Homo sapiens] >pir|S29127|S29127 carboxypeptidase A (EC 3.4.17.1) CPA1 precursor - human >sp|P15085|CBP1_HUMAN CARBOXYPEPTIDASE A1 PRECURSOR (EC 3.4.17.1). Length = 419	gnl|PID|e307065	1	255	97	97	HTPDO06
58	598665	unnamed protein product [unidentified] >gi|35330 carboxypeptidase a [Homo sapiens] >pir|S29127|S29127 carboxypeptidase A (EC 3.4.17.1) CPA1 precursor - human >sp|P15085|CBP1_HUMAN CARBOXYPEPTIDASE A1 PRECURSOR (EC 3.4.17.1). Length = 419	gnl|PID|e307065	1	1218	100	100	HTPEE79
59	604719	ADP-ribosylation factor [Bos taurus] >gi|178156 ADP-ribosylation factor 1 [Homo sapiens] >gi|178164 ADP-ribosylation factor 1 [Homo sapiens]	gi|162627	1	651	100	100	HSHCL62
60	612689			243	578			HMADQ02
61	612980			47	259			HISAD74
62	615134	metavinculin - pig (fragments) >gi|2283 metavinculin [Sus scrofa] {SUB 113-336} Length = 336	pir|S29507|S29507	30	578	100	100	HAHEM20
63	616064			159	398			HASCD63

64	616096	(AC004877) sco-spondin-mucin-like; similar to P98167 (PID:g1711548); details of intron/exon structure uncertain [Homo sapiens] >sp|O75851|O75851 WUGSC:H_DJ0751H13.1 PROTEIN (FRAGMENT). Length = 4123	gi|3638957	99	221	40	52	HOHCQ05
65	616926	Gps2 [Homo sapiens] Length = 327	gi|1049070	2	1123	100	100	HDPJK81
66	634923	islet regenerating protein [Homo sapiens] >pir|A35197|RGHU1A regenerating islet lectin 1-alpha precursor - human >sp|P05451|LITA_HUMAN LITHOSTATHINE 1 ALPHA PRECURSOR (PANCREATIC STONE PROTEIN) (PSP) (PANCREATIC THREAD PROTEIN) (PTP) (ISLET OF LANGERHANS	gi|190979	148	447	81	88	HTPBT17
67	646688	ORF1 [Homo sapiens] >sp|Q14921|Q14921 NONSPECIFIC CROSSREACTING ANTIGEN. Length = 100	gi|189086	508	819	99	99	HTPDY03
68	647531	calcium-dependent protease [Oryctolagus cuniculus] >pir|B24815|B24815 calpain (EC 3.4.22.17) large chain 2 - rabbit (fragment) >sp|P06814|CAN2_RABIT CALPAIN 2, LARGE [CATALYTIC] SUBUNIT (EC 3.4.22.17) (CALCIUM-ACTIVATED NEUTRAL PROTEINASE) (CANP) (M-TYPE)	gi|165666	809	1177	76	81	HMSCC36

69	647695	preprocarboxypeptidase A2 [Homo sapiens] >pir|A56171|A56171 carboxypeptidase A2 (EC 3.4.17.15) precursor - human >sp|P48052|CPA2_HUMAN CARBOXYPEPTIDASE A2 PRECURSOR (EC 3.4.17.15). Length = 417	gi|790227	2	1285	92	92	HVAAB38
70	647699	chymotrypsin-like protease CTRL-1 [Homo sapiens] >gi|406228 chymotrypsin-like protease CTRL-1 [Homo sapiens] >pir|I38136|I38136 chymotrypsin-like proteinase (EC 3.4.21.-) CTRL-1 - human >sp|P40313|CTRL_HUMAN CHYMOTRYPSIN-LIKE PROTEASE CTRL-1 PRECURSOR (EC	gi|438039	2	577	100	100	HCCMB81
71	651706	erythrocyte p55 [Homo sapiens] >sp|Q00013|EM55_HUMAN 55 KD ERYTHROCYTE MEMBRANE PROTEIN (P55). Length = 466	gi|189786	1	963	96	97	HTHCO11
72	651726	arylacetamide deacetylase [Homo sapiens] >pir|A53856|A53856 aryl-acylamidase (EC 3.5.1.13) - human >sp|P22760|AAAD_HUMAN ARYLACETAMIDE DEACETYLASE (EC 3.1.1.-) (AADAC). {SUB 2-399} Length = 399	gi|537514	384	1553	99	99	HFVHL70
73	652160	alpha 2-macroglobulin 690-730 [Homo sapiens] Length = 1474	gi|579592	78	860	92	92	HDPUB04
74	654015			172	390			HSAV29

75	656339	alpha endosulfine [Homo sapiens] >sp|O43768|O43768 ALPHA ENDOSULFINE. >gnl|PID|e224652 alpha endosulfine [Bos taurus] {SUB 25-101} Length = 121	gnl|PID|e284090	1	450	100	100	HKGCM36
76	657190			293	493			HLTHI43
77	657859			3	323			HNKAA14
78	662143			576	722			HLDQI35
79	662212	FK506 polyketide synthase [Streptomyces sp.] >sp|P95814|P95814 FK506 POLYKETIDE SYNTHASE. Length = 6420	gnl|PID|e290681	11	457	45	59	HTPAG88
80	662225			107	289			HWACN48
81	662496			3	446			HWHHG17
82	669529			254	343			HSBT20
83	670453	acid sphingomyelinase [Homo sapiens] >sp|Q16837|Q16837 ACID SPHINGOMYELINASE (EC 3.1.4.12) (SPHINGOMYELIN PHOSPHODIESTERASE) (NEUTRAL SPHINGOMYELINASE). >gi|972770 acid sphingomyelinase [Homo sapiens] {SUB 33-629} Length = 629	gi|972769	926	1648	100	100	HMAJQ46
84	675028	seven in absentia homolog [Homo sapiens] >gi|2673966 hSIAH1 [Homo sapiens] >sp|O43269|O43269 HSIAH1. Length = 282	gi|3041825	18	284	100	100	HE2IJ09
85	681325			3	224			HAJBC26
86	683103			212	1024			HTTBN65

87	684432	serine hydroxymethyltransferase [Homo sapiens] >gi|307422 serine hydroxymethyltransferase [Homo sapiens] >pir|A46746|A46746 glycine hydroxymethyltransferase (EC 2.1.2.1), cytosolic - human >sp|P34896|GLYC_HUMAN SERINE HYDROXYMETHYLTRANSFERASE, CYTOSOLIC (	gi|438636	3	905	94	95	HSPAA79
88	688018	protease (put.); putative [Simian immunodeficiency virus] >sp|Q85727|Q85727 PIGTAILED MONKEY SIMIAN T-CELL LEUKEMIA VIRUS PROTEASE (FRAGMENT). Length = 215	gi|334735	169	351	66	77	HTPDE05
89	688077	(AF047440) ribosomal protein L33-like protein [Homo sapiens] >sp|O75394|O75394 RIBOSOMAL PROTEIN L33-LIKE PROTEIN. Length = 65	gi|3335136	1	276	100	100	HEBAG86
90	691522	similar to vacuolar biogenesis protein (pen5); cDNA EST EMBL:D27614 comes from this gene; cDNA EST EMBL:D34974 comes from this gene [Caenorhabditis elegans] >gnl|PID|e1351725 similar to vacuolar biogenesis protein (pep5); cDNA EST EMBL:D27614 comes from t	gnl|PID|e1351725	1	1179	32	54	HTACN89

91	693706	transcription factor ISGF-3 [Homo sapiens] >sp|G2281071|G2281071 TRANSCRIPTION FACTOR ISGF-3. >sp|G1168081|G1168081 SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1A, STAT1A=INTERLEUKIN-6 RESPONSE ELEMENT BINDING PROTEIN {SRC-HOMOLOGY DOMAIN TYPE 2}. {S	gi|2281071	184	2541	98	98	HAICK36
92	694523			1	204			HTPDK30
93	697517	PHOSPHOGLUCOMUTASE (EC 5.4.2.2) (GLUCOSE PHOSPHOMUTASE) (PGM). Length = 561	sp|P36871|PGMU_HUMAN	117	1649	91	91	HBDL34
94	699054			338	889			HMECT29
95	699464			2	241			HTLDA59
96	703402	(AF000422) TTF-I interacting peptide 5 [Homo sapiens] >sp|O00536|O00536 TTF-I INTERACTING PEPTIDE 5 (FRAGMENT). Length = 407	gi|2183083	1	423	94	95	HHFHV36
97	703651	guanine nucleotide-binding protein alpha subunit, G alpha 12 - human >sp|G264227|G264227 GUANINE NUCLEOTIDE-BINDING PROTEIN ALPHA SUBUNIT, G ALPHA 12. Length = 381	pir|A48071|A48071	3	332	99	99	HDPGN16
98	704905			359	655			HPMFK19
99	706907			1	153			HRSAR67

100	708515	CDM [Homo sapiens] >gi|535058 tumor-associated antigen [Homo sapiens] >gi|1508820 CDM protein [Homo sapiens] >pir|S44279|S44279 CDM protein - human >sp|P51572|CDM_HUMAN CDM PROTEIN (6C6-AG TUMOR-ASSOCIATED ANTIGEN) (DXS1357E). >gi|1020320 CDM protein [Hom	gi|479157	1	480	87	87	HCFDA53
101	710572			6	248			HHASC40
102	710618			125	325			HBMAC72
103	711810			283	543			HBXCZ83
104	714933	RNA adenosine deaminase [Homo sapiens] >sp|O43859|O43859 RNA ADENOSINE DEAMINASE. Length = 1181	gi|2795790	2	370	89	90	HAMFQ09
105	716331	(AF006621) embryonic lung protein [Homo sapiens] >sp|G2654559|G2654559 EMBRYONIC LUNG PROTEIN. Length = 568	gi|2654559	1	564	91	91	HLTHP10
106	717686			282	494			HTPBX62
107	718187			1	153			HTPDG49
108	719934	triglyceride lipase precursor [Homo sapiens] >gi|190140 lipase [Homo sapiens] >gi|1304379 pancreatic lipase [Homo sapiens] >pir|C43357|C43357 triacylglycerol lipase (EC 3.1.1.3) precursor, pancreatic - human >sp|P16233|LIPP_HUMAN TRIACYLGLYCEROL LIPASE PR	gi|339597	2	1420	100	100	HPASD23

109	722980	plasma gelsolin precursor [Sus scrofa] >gi|758306 gelsolin [Sus scrofa] >pir|S02665|S02665 gelsolin precursor - pig (fragment) >sp|P20305|GELS_PIG GELSOLIN PRECURSOR, PLASMA (ACTIN-DEPOLYMERIZING FACTOR) (ADF) (BREVIN) (FRAGMENT). Length = 772	gi|164472	67	375	100	100	HLQCT60
110	723596	poly(A) binding protein [Mus musculus] >pir|I48718|I48718 poly(A) binding protein - mouse >sp|P29341|PAB1_MOUSE POLYADENYLATE-BINDING PROTEIN 1 (POLY(A) BINDING PROTEIN 1) (PABP 1). Length = 636	gi|53754	1010	1225	61	67	HTTDR30
111	724352			255	662			HKGBC30
112	724450			311	547			HMWGX50
113	724855			3	440			HJPBC80
114	724904	(AJ225089) 2'-5' oligoadenylate synthetase (p59OAS) [Homo sapiens] >sp|O75686|O75686 2'-5' OLIGOADENYLATE SYNTHETASE (P59OAS). Length = 514	gnl|PID|e1316607	1	417	100	100	HT3TB04
115	725642	synaptotagmin VI [Rattus norvegicus] >pir|S58399|S58399 cellutagmin I sytVI - rat >sp|Q62746|Q62746 SYNAPTOTAGMIN VI. Length = 511	gi|643654	1	303	85	86	HSBX52
116	726192	Highly similar to murine eps 15 GB A.N. L221768 [Homo sapiens] >pir|S43074|S43074 AF-1p protein - human Length = 896	gi|470035	276	785	79	79	HSKXH03

117	726964	elastase 2 precursor [Homo sapiens] >gi|182058 pancreatic elastase IIA zymogen [Homo sapiens] >pir|B26823|B26823 pancreatic elastase II (EC 3.4.21.71) A precursor - human >sp|P08217|EL2A_HUMAN ELASTASE 2A PRECURSOR (EC 3.4.21.71). Length = 269	gi|182023	2	808	92	92	HPASF77
118	730930	glutathione synthetase [Homo sapiens] >gi|1236350 glutathione synthetase [Homo sapiens] >pir|S56748|S56748 glutathione synthase (EC 6.3.2.3), brain - human >sp|P48637|GSHB_HUMAN GLUTATHIONE SYNTHETASE (EC 6.3.2.3) (GLUTATHIONE SYNTHASE) (GSH SYNTHETASE) (	gi|886284	22	1530	97	97	HLWBL10
119	731314			91	261			HSPAK79
120	732386			3	632			HLTDQ55
121	732909			759	1220			HLTCA26
122	733088	(AF029786) GBAS [Homo sapiens] >sp|O75323|O75323 GBAS. Length = 286	gi|3403167	1	399	100	100	HADEY44
123	733351			287	463			HDPCL56
124	733693			1	276			HTPDU59
125	734760	(AF042379) spindle pole body protein spc97 homolog GCP2 [Homo sapiens] >sp|O43632|O43632 SPINDLE POLE BODY PROTEIN SPC97 HOMOLOG. Length = 902	gi|2801701	205	1584	96	96	HEMEU68
126	735711	glutamate pyruvate transaminase [Homo sapiens] Length = 496	gi|1763096	12	842	66	82	H2CBI61

127	742413			3	779			HFTAS62
128	742676	common fibrinogen alpha chain [Homo sapiens] >gi|182426 A-alpha fibrinogen [Homo sapiens] >gi|4033511 fibrinogen alpha subunit [Homo sapiens] >pir|A93956|FGHUA fibrinogen alpha chain precursor, short splice form - human >gi|532482 alpha-fibrinogen [Homo s	gi|458554	2	1081	89	89	HLICN22
129	742781			344	607			HE8AY14
130	743356			173	298			HCFDA89
131	745694			1	105			HETHK36
132	747235			63	743			HEBFI18
133	750986	syntaxin 7 [Homo sapiens] Length = 261	gi|2337920	156	689	83	83	HLQCR21
134	751068			529	810			HRABQ88
135	751164			1341	1736			HCE3G96
136	751890			18	308			HTPBR05
137	751991			1	1530			HMSIS33
138	752449			1	270			HLYDM55
139	752504	pro-alpha-1 type V collagen [Homo sapiens] >pir|S18802|CGHU1V collagen alpha 1(V) chain precursor - human >sp|Q15094|Q15094 PRO-ALPHA-1 TYPE V COLLAGEN. Length = 1838	gi|189520	1	552	94	95	HOUDR20

140	752688	(AF006088) p16-Arc [Homo sapiens] >gi|2407611 (AF017807) Arp2/3 complex 16kDa subunit [Homo sapiens] >sp|O15511|AR16_HUMAN ARP2/3 COMPLEX 16 KD SUBUNIT (P16-ARC). Length = 151	gi|2282042	140	451	100	100	HEBGK82
141	752889	testican [Homo sapiens] >sp|Q08629|Q08629 TESTICAN PRECURSOR. >gi|3282168 (AC005213) testican [Homo sapiens] {SUB 237-439} Length = 439	gi|793845	153	518	87	87	HE8DN77
142	753150	pre-mRNA splicing factor [Homo sapiens] >pir|A48133|A48133 pre-mRNA splicing SRp75 - human >gi|2914669 (AC004236) SRP0001LB [Homo sapiens] {SUB 1-192} Length = 494	gi|307438	259	1176	100	100	HELHM06
143	753690			246	743			HHPBG69
144	754479			160	543			HISDF69
145	754692	(AL031058) dJ512B11.1 (Desmoplakin I (DPI)) [Homo sapiens] >sp|O75993|O75993 DJ512B11.1 (DESMOPLAKIN I (DPI)). Length = 2871	gnl|PID|e1329910	338	2122	87	87	HTTBL33

146	756814	glucose transporter glycoprotein [Homo sapiens] >pir|A27217|A27217 glucose transport protein - human >sp|P11166|GTR1_HUMAN GLUCOSE TRANSPORTER TYPE 1, ERYTHROCYTE/BRAIN. >bbs|77925 glucose transporter isoform 1, GLUT 1 [mice, embryo, Peptide Partial, 107	gi|183303	44	679	90	90	HCWBT85
147	757127			253	501			HBAFN70
148	757347			213	620			HMSFX70
149	757495	(AJ010046) guanine nucleotide-exchange factor [Homo sapiens] >sp|E1363645|E1363645 GUANINE NUCLEOTIDE-EXCHANGE FACTOR. Length = 548	gnl|PID|e1363645	88	1221	99	100	HTPBY44
150	757715	(AL008986) similar to tyrosine-protein kinase [Caenorhabditis elegans] >gnl|PID|e1348186 similar to tyrosine-protein kinase [Caenorhabditis elegans] >sp|O45668|O45668 H37N21.1 PROTEIN. Length = 231	gnl|PID|e1347680	227	1729	72	84	HEBFD01
151	760388			2	232			HLWAH26
152	760433	mutant sterol regulatory element binding protein-2 [Cricetulus griseus] Length = 839	gi|841318	2	730	83	89	HJBDG86
153	760545	(AF051426) slow delayed rectifier channel subunit [Homo sapiens] >sp|O60607|O60607 SLOW DELAYED RECTIFIER CHANNEL SUBUNIT. Length = 548	gi|2961249	1	345	80	81	HSSFS06

154	761566	protein tyrosine phosphatase PTPCAAX1 [Homo sapiens] >gi|2961199 (AF051160) tyrosine phosphatase [Homo sapiens] >gi|530162 tyrosine phosphatase [Rattus rattus] >gi|1814024 protein tyrosine phosphatase [Mus musculus] >pir|A56059|A56059 protein-tyrosine-pho	gi|1777755	601	1125	99	99	HDPOW14
155	761740			7	435			HSIFY01
156	765215	colipase precursor [Homo sapiens] >gi|1483624 colipase [Homo sapiens] >pir|A42568|XLHU colipase precursor - human >sp|P04118|COL_HUMAN COLIPASE PRECURSOR. Length = 112	gi|180886	25	417	86	86	HTPCY18
157	765428			142	627			HLYCF90
158	766686			38	901			HNTNP54
159	767396	(AF050640) NADH-ubiquinone oxidoreductase NDUFS2 subunit [Homo sapiens] >sp|G3337443|G3337443 NADH-UBIQUINONE OXIDOREDUCTASE NDUFS2 SUBUNIT. Length = 463	gi|3337443	2	568	87	89	HSKNG05
160	767501	L-arginine:glycine amidinotransferase {EC 2.1.4.1} [human, kidney carcinoma cells, Peptide, 423 aa] [Homo sapiens] >pir|S41734|S41734 glycine amidinotransferase (EC 2.1.4.1) precursor - human >sp|P50440|GATM_HUMAN GLYCINE AMIDINOTRANSFERASE PRECURSOR (EC	bbs|143982	1	735	95	95	HKMMB02

161	767945			183	302			HSEB76
162	768996			209	454			HTPCQ34
163	771415			1	747			HTENW77
164	772657	zyginII [Rattus norvegicus] >sp|P97578|P97578 ZYGINII (FRAGMENT). Length = 324	gi|1778078	110	1087	69	72	HDQEV69
165	773123	beta-polymerase [Homo sapiens] >gi|553614 polymerase beta [Homo sapiens] {SUB 1-39} Length = 335	gi|190156	278	1012	35	58	HWHHN55
166	773193			246	695			HBODO78
167	773710			3	707			HFIYK62
168	774283	(AF047384) postsynaptic protein CRIPT [Rattus norvegicus] >sp|O70333|O70333 POSTSYNAPTIC PROTEIN CRIPT. Length = 101	gi|3098551	3	395	99	99	HATCI32
169	774369			33	224			HDAAC66
170	774754	(AF013758) polyadenylate binding protein-interacting protein-1 [Homo sapiens] >sp|O60455|O60455 POLYADENYLATE BINDING PROTEIN-INTERACTING PROTEIN-1. Length = 480	gi|3046900	52	1239	92	92	HE8ON42
171	774823	out at first [Drosophila virilis] >sp|O18638|O18638 OUT AT FIRST. Length = 305	gi|2443448	2	568	46	62	HMKEG85
172	775510			3	98			HVAMK80
173	775634			106	537			HTXBI91
174	775640			53	358			HTWDN88
175	775802			762	971			HJMAF44

176	777470	Ner-1 [Homo sapiens] >pir|JC4014|JC4014 steroid hormone-nuclear receptor NER - human >sp|P55055|NER_HUMAN NUCLEAR RECEPTOR NER (UBIQUITOUSLY-EXPRESSED NUCLEAR RECEPTOR). >gi|608135 orphan receptor [Homo sapiens] {SUB 7-461} Length = 461	gi|641962	731	1621	91	91	HTACM37
177	777652			2	340			HDPVH10
178	778998			197	409			HSCOI0
179	779273	(AF053091) eyelid [Drosophila melanogaster] >sp|O61603|O61603 EYELID. Length = 2715	gi|2981221	1	699	51	67	HRADK51
180	779297	cek5 receptor ligand [Mus musculus] Length = 345	gi|575929	1	318	100	100	HCEBB53
181	779664	enhancer-trap-locus-1 [Mus musculus] >pir|A56559|A56559 enhancer-trap-locus-1 protein - mouse (fragment) >sp|Q04692|Q04692 ENHANCER TRAP LOCUS 1 (ENHANCER-TRAP-LOCUS 1 PROTEIN) (FRAGMENT). Length = 1136	gi|50866	184	675	98	99	HSCI82
182	780565			10	144			HSDJ93
183	780665	preprochymotrypsinogen (EC 3.4.21.1) [Homo sapiens] >pir|A31299|A31299 chymotrypsin (EC 3.4.21.1) precursor - human >sp|P17538|CTRB_HUMAN CHYMOTRYPSINOGEN B PRECURSOR (EC 3.4.21.1). Length = 263	gi|181190	3	401	100	100	HVANF29

184	780666	preprochymotrypsinogen (EC 3.4.21.1) [Homo sapiens] >pir|A31299|A31299 chymotrypsin (EC 3.4.21.1) precursor - human >sp|P17538|CTRB_HUMAN CHYMOTRYPSINOGEN B PRECURSOR (EC 3.4.21.1). Length = 263	gi|181190	57	494	91	93	HTPDP51
185	781579	26S proteasome regulatory chain 12 - human Length = 321	pir|S65491|S65491	56	1102	94	94	HLWBI70
186	782052	(AP000002) 376aa long hypothetical dehydrogenase [Pyrococcus horikoshii] >sp|O58320|O58320 376AA LONG HYPOTHETICAL DEHYDROGENASE. Length = 376	gnl|PID|d1030629	76	447	35	58	HSDQ77
187	782393			3	269			HSEC84
188	782907			2	379			HSSFR96
189	783220			273	635			HFXJX12
190	783300	(AF068195) putative glialblastoma cell differentiation-related protein [Homo sapiens] >sp|O75500|O75500 PUTATIVE GLIALBLASTOMA CELL DIFFERENTIATION-RELATED PROTEIN. Length = 334	gi|3211975	685	1392	86	86	HDPIZ33
191	783938			1139	1495			HTDAE52
192	784024			355	540			HMEIS41
193	784575			615	806			HSAC93
194	785006			3	353			HSDH86
195	785069			3	206			HDTAT56
196	785237			46	132			HDTFQ86
197	786111			2	331			HTPCQ24

198	787036	(AL008583) dJ327J16.1 (human ortholog of mouse outer arm Dynein light chain 4) [Homo sapiens] Length = 105	gnl|PID|e1370730	123	518	100	100	HSSAL37
199	788991			74	556			HCLBH73
200	789125			3	515			HTXLS64
201	789626			98	742			HFPBL42
202	789703			2	817			HDPJU62
203	789858			351	806			HATEH30
204	790848			891	1067			HTPDK53
205	790893			29	244			HFVIE06
206	790912			293	544			HISET09
207	791386	ADP-ribosylation factor 1-directed GTPase activating protein [Rattus norvegicus] >sp|Q62848|Q62848 ADP-RIBOSYLATION FACTOR 1-DIRECTED GTPASE ACTIVATING PROTEIN. Length = 415	gi|1130494	1	366	73	84	HDQFB29
208	791598			399	644			HOEDP59
209	791619			1	1698			HOGCS94
210	791628			125	466			HOEBI96
211	791751			3	464			HE8OL02
212	792557	(AB004066) DEC1 [Homo sapiens] >pir|JC5547|JC5547 basic helix-loop-helix factor DEC1 - human >sp|O14503|O14503 DEC1. Length = 412	gnl|PID|d1022575	231	470	92	93	HAMFQ15

213	792568	unknown [Saccharomyces cerevisiae] >pir|S53571|S53571 hypothetical protein YIL128w - yeast (Saccharomyces cerevisiae) >sp|P40469|MT18_YEAST DNA REPAIR/TRANSCRIPTION PROTEIN MET18/MMS19. >gi|599989 unknown [Saccharomyces cerevisiae] {SUB 162-1032} Length =	gi|763218	211	882	36	58	HUVDR47
214	792590	O-linked GlcNAc transferase [Homo sapiens] >sp|O15294|O15294 UDP-N-ACETYLGLUCOSAMINE--PEPTIDE N-ACETYLGLUCOSAMINYLTRANSFERASE 100 KD SUBUNIT (EC 2.4.1.-) (O-GLCNACTRANSFERASE P100 SUBUNIT). Length = 920	gi|2266994	1163	2656	100	100	HTTCQ93
215	793323			2	439			HBWBJ67
216	793466			567	887			HHBCH37
217	793507			634	903			HAGER01
218	793546			3	1088			H2CBU95
219	793559			137	295			HISBT02
220	793604	(AB008430) CDEP [Homo sapiens] >sp|D1025178|D1025178 CDEP. Length = 1045	gnl|PID|d1025178	3	1187	96	96	HISBR20
221	794121			2	469			HOEAN65
222	794295			2	433			HFIAA16
223	795241	F46F6.1 (FRAGMENT). Length = 509	sp|Q20473|Q20473	556	1089	43	67	HOEDV07
224	795286			132	1487			HTJNQ55
225	795637			2	1282			HHBHM95

226	796301	(AF053367) carboxyl terminal LIM domain protein [Mus musculus] >sp|O70400|O70400 CARBOXYL TERMINAL LIM DOMAIN PROTEIN. Length = 326	gi|2996196	142	1104	63	82	HKACQ38
227	796347			740	952			HOGCR67
228	796579	U2 small nuclear ribonucleoprotein B" [Homo sapiens] >pir|A25910|A25910 small nuclear ribonucleoprotein U2B" - human Length = 225	gi|340105	32	847	88	88	HOGAQ65
229	796590	(AF039700) antigen NY-CO-38 [Homo sapiens] >sp|G3170200|G3170200 ANTIGEN NY-CO-38. >gi|3170198 (AF039699) antigen NY-CO-37 [Homo sapiens] {SUB 1-403} Length = 652	gi|3170200	54	572	41	65	HTXBD96
230	799783			1	198			HPNAA04
231	799784			1	282			HTPCY49
232	799785			176	373			HTPCW69
233	799786			26	397			HISEL18
234	799787			78	245			HTPDR86
235	799800			1	210			HTPDJ82
236	799808	pancreatic protease E precursor [Homo sapiens] >sp|P09093|EL3A_HUMAN ELASTASE IIIA PRECURSOR (EC 3.4.21.70) (PROTEASE E). Length = 270	gnl|PID|d1000660	2	820	97	97	HCCMD30

237	799977	(AJ000342) DMBT1 protein. 5.8 kb transcript [Homo sapiens] >sp|E328724|E328724 DMBT1 PROTEIN. 5.8 KB TRANSCRIPT PRECURSOR. Length = 1785	gnl|PID|e328724	2	541	81	81	HTPDX19
238	800149	(AB017365) frizzled-7 [Homo sapiens] >sp|D1035649|D1035649 FRIZZLED-7. Length = 574	gnl|PID|d1035649	3	188	96	96	HSBC04
239	800189	NTGP4 [Nicotiana tabacum] >sp|G4097585|G4097585 NTGP4 (FRAGMENT). Length = 344	gi|4097585	33	434	45	68	HLDDQ25
240	800589	retinal-specific heterotrimeric GTP-binding protein beta subunit, G beta5L [Mus musculus] >sp|G1663629|G1663629 RETINAL-SPECIFIC HETEROTRIMERIC GTP-BINDING PROTEIN BETA SUBUNIT, G BETA5L. >gi|557738 guanine nucleotide regulatory protein [Mus musculus] {SU	gi|1663629	137	1249	99	100	HARAG68
241	800811	SH3-domain interacting protein [Homo sapiens] >sp|Q15220|Q15220 PRPL-2 PROTEIN. >pir|S52796|S52796 prpL2 protein - human (fragment) {SUB 92-494} Length = 494	gnl|PID|e1226443	2	601	100	100	HSKGN39
242	800857	p78 protein [Homo sapiens] >sp|P20591|MX1_HUMAN INTERFERON-REGULATED RESISTANCE GTP-BINDING PROTEIN MXA (INTERFERON- INDUCED PROTEIN P78) (IFI-78K). {SUB 2-662} Length = 662	gi|190136	295	2274	96	96	HMTBX80
243	805721			3	224			HSKYW73
244	805818			143	994			HJABT11

245	806267	(AF022982) contains similarity to the A-type potassium current class of channel proteins [Caenorhabditis elegans] >sp|O17001|O17001 T23B12.6 PROTEIN. Length = 670	gi|2384910	2	1018	57	75	HDTDM49
246	806579			971	1282			HASC33
247	810625	(AF085691) multidrug resistance-associated protein 3A [Homo sapiens] >sp|G4106442|G4106442 MULTIDRUG RESISTANCE-ASSOCIATED PROTEIN 3A. Length = 1238	gi|4106442	3	1019	96	96	HWFAD06
248	811153	SHB=SRC HOMOLOGY 2 PROTEIN. Length = 309	sp|G545100|G545100	531	881	83	96	HDQED71
249	811787			1201	1797			HEONN51
250	812314	ubiquitin-specific protease [Drosophila melanogaster] Length = 898	gnl|PID|e252797	648	1514	46	67	HUSXO71
251	812443	binding factor-2 box B [Drosophila melanogaster] >pir|A42140|A42140 box B-binding factor-2 - fruit fly (Drosophila melanogaster) >sp|P29747|BBF2_DROME BOX B BINDING FACTOR-2 (BBF-2). Length = 515	gi|11064	2	733	67	85	HE8PW90
252	812498			1290	1550			HE8PW45
253	812504	(AF035737) general transcription factor 2-I; alternative splice product [Homo sapiens] >sp|O43546|O43546 GENERAL TRANSCRIPTION FACTOR 2-I. ALTERNATIVE SPLICE PRODUCT. Length = 998	gi|2827180	3	1601	98	99	HFIAT66

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<tr><td>254</td><td>813079</td><td>GS2NA [Homo sapiens] >pir|JC2522|JC2522 nuclear autoantigen - human Length = 713</td><td>gi|805095</td><td>1</td><td>756</td><td>62</td><td>73</td><td>HCROB17</td></tr>
<tr><td>255</td><td>815889</td><td>centrin [Homo sapiens] >sp|O15182|O15182 CENTRIN. Length = 167</td><td>gnl|PID|e314005</td><td>1</td><td>603</td><td>99</td><td>100</td><td>HOEFN43</td></tr>
<tr><td>256</td><td>824358</td><td>oxysterol-binding protein [Homo sapiens] >pir|A34581|A34581 oxysterol-binding protein - human >sp|P22059|OXYB_HUMAN OXYSTEROL-BINDING PROTEIN. Length = 807</td><td>gi|189403</td><td>415</td><td>1275</td><td>100</td><td>100</td><td>HMSK174</td></tr>
<tr><td>257</td><td>826144</td><td>ets-related protein [Homo sapiens] >gnl|PID|e225719 erm [Homo sapiens] >pir|S43692|S43692 transcription factor erm - human >sp|P41161|ERM_HUMAN ETS-RELATED PROTEIN ERM (ETS TRANSLOCATION VARIANT 5). Length = 510</td><td>gi|479167</td><td>1</td><td>609</td><td>100</td><td>100</td><td>HNFJH73</td></tr>
<tr><td>258</td><td>826558</td><td></td><td></td><td>3</td><td>266</td><td></td><td></td><td>HTAFE67</td></tr>
<tr><td>259</td><td>827471</td><td>cholesterol esterase [Homo sapiens] >bbs|109185 pancreatic cholesterol esterase, CEase {internal fragment} {EC 3.1.1.13} [human, pWE 15, PTCF, Peptide Partial, 28 aa] [Homo sapiens] {SUB 458-485} Length = 747</td><td>gi|180482</td><td>1</td><td>1077</td><td>96</td><td>96</td><td>HVANU76</td></tr>
<tr><td>260</td><td>827716</td><td>(AF008197) syncollin [Rattus norvegicus] >sp|O35775|O35775 SYNCOLLIN. >gi|3366638 (AF012887) sip9 [Rattus norvegicus] {SUB 8-145} Length = 145</td><td>gi|2258437</td><td>2</td><td>403</td><td>72</td><td>84</td><td>HVANR45</td></tr>
<tr><td>261</td><td>827722</td><td></td><td></td><td>1976</td><td>2116</td><td></td><td></td><td>HISCW21</td></tr>
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262	827727			1192	1557			HSCC19
263	828238	(AC002451) pyruvate dehydrogenase kinase isoform 4 [Homo sapiens] >gi|1399197 pyruvate dehydrogenase kinase isoform 4 [Homo sapiens] >gi|1399210 pyruvate dehydrogenase kinase isoform 4 [Homo sapiens] >sp|Q16654|PDK4_HUMAN [PYRUVATE DEHYDROGENASE(LIPOAMIDE	gi|2337883	2	301	100	100	HLTGU39
264	828573			3	1118			HGCAA50
265	828624			1305	1520			HTOEU64
266	828656			185	403			HSDJ27
267	828848	pancreatic zymogen granule membrane protein GP-2 [Homo sapiens] >pir|G02091|G02091 pancreatic zymogen granule membrane protein GP-2 - human >sp|P55259|GP2_HUMAN PANCREATIC SECRETORY GRANULE MEMBRANE MAJOR GLYCOPROTEIN GP2 PRECURSOR (PANCREATIC ZYMOGEN GRA	gi|1244512	3	1220	97	97	HVANS09
268	828929	casein kinase I alpha L [Rattus norvegicus] >sp|P97634|P97634 CASEIN KINASE I ALPHA L. >gi|975691 casein kinase I-alpha [Mus musculus] {SUB 327-353} Length = 353	gi|1679790	3	479	80	80	HMICG83
269	829008	GTP-binding protein [Homo sapiens] >sp|O43824|O43824 GTP-BINDING PROTEIN. Length = 442	gnl|PID|e1227622	17	694	80	81	HSAH79

270	829086	small GTP-binding protein [Oryctolagus cuniculus] >pir|A48500|A48500 small GTP-binding protein Rab25 - rabbit Length = 213	gi|436001	244	426	82	87	HTPDE66
271	829192	pLK [Homo sapiens] Length = 603	gi|393017	455	1270	94	95	HMEKC67
272	829310	SUP35 gene product [Xenopus laevis] >pir|S58444|S58444 SUP35 protein - African clawed frog (fragment) Length = 614	gi|976219	3	527	90	95	HAMFY36
273	829319			407	805			HUFDB42
274	829459	spermatid-specific [Mus musculus] >pir|A37363|A37363 histone H2B, testis - mouse (fragment) >sp|Q64477|Q64477 HISTONE H2B (FRAGMENT). Length = 134	gi|556310	2	184	97	100	HNFAA17
275	829527			489	806			HTTDP69
276	829736	(AL031532) yeast gtr2 homolog, novel small GTPase subfamily protein [Schizosaccharomyces pombe] >sp|O74544|O74544 YEAST GTR2 HOMOLOG, NOVEL SMALL GTPASE SUBFAMILY PROTEIN. Length = 314	gnl|PID|e1319429	3	1049	69	86	HTGCM10
277	830552			329	1732			HTTEV24

278	830566	cathepsin E precursor [Homo sapiens] >gi|181205 cathepsin E [Homo sapiens] >pir|A42038|A34401 cathepsin E (EC 3.4.23.34) precursor - human >sp|P14091|CATE_HUMAN CATHEPSIN E PRECURSOR (EC 3.4.23.34). >sp|G402841|G402841 CATHEPSIN E, CE=MATURE FORM {N-TERMI	gi|181194	1	552	99	100	HTPBQ32
279	830568	tyrosine protein kinase [Homo sapiens] >sp|Q08345|EDD1_HUMAN EPITHELIAL DISCOIDIN DOMAIN RECEPTOR 1 PRECURSOR (EC 2.7.1.112) (TYROSINE-PROTEIN KINASE CAK) (CELL ADHESION KINASE) (TYROSINE KINASE DDR) (DISCOIDIN RECEPTOR TYROSINE KINASE) (TRK E) (PROTEIN-T	gi|306475	3	1874	95	95	HTSGO78
280	830569	lipase related protein 2 [Homo sapiens] >pir|B43357|B43357 pancreatic lipase-related protein 2 - human >sp|P54317|LIP2_HUMAN PANCREATIC LIPASE RELATED PROTEIN 2 PRECURSOR (EC 3.1.1.3). Length = 469	gi|187232	3	1433	98	98	HSIAL52
281	830583	alpha-tropomyosin 5b [Rattus norvegicus] >pir|D39816|D39816 tropomyosin 5b, fibroblast - rat >sp|Q63609|Q63609 ALPHA-TROPOMYOSIN 5B. Length = 248	gi|207508	1	909	85	85	HAROA79

282	830613	clathrin-associated protein [Mus musculus] >pir|S19693|S19693 AP47 protein - mouse >sp|P35585|AP47_MOUSE CLATHRIN COAT ASSEMBLY PROTEIN AP47 (CLATHRIN COAT ASSOCIATED PROTEIN AP47) (GOLGI ADAPTOR AP-1 47 KD PROTEIN) (HA1 47 KD SUBUNIT) (CLATHRIN ASSEMBLY	gi|191986	3	1091	77	90	HETFJ47
283	830686			197	391			HSTAH26
284	830691			1343	1627			HSXEO71
285	830716	(AL031393) dJ733D15.1 (Zinc-finger protein) [Homo sapiens] Length = 496	gnl|PID|e1329909	3	713	62	74	HSSET42
286	830792	kallikrein [Homo sapiens] >pir|A24696|KQHU tissue kallikrein (EC 3.4.21.35) precursor - human >sp|P06870|KLK1_HUMAN GLANDULAR KALLIKREIN 1 PRECURSOR (EC 3.4.21.35) (TISSUE KALLIKREIN) (KIDNEY/PANCREAS/SALIVARY GLAND KALLIKREIN). >gi|386843 kallikrein [Hom	gi|186653	1	801	99	99	HSDSG96
287	830893			29	535			HPRTG34
288	830976	(AF077866) amino acid transporter E16 [Homo sapiens] >sp|G3639058|G3639058 AMINO ACID TRANSPORTER E16. >gi|181908 E16 [Homo sapiens] {SUB 267-507} Length = 507	gi|3639058	3	1244	53	73	HDPRH64

289	831043	adhesive protein - mussel (Trichomya hirsuta) (fragments) Length = 65	pir|S42675|S42675	24	257	41	62	HOABZ73
290	831131			23	301			HMTBI28
291	831164			59	331			HMSGB46
292	831173	pancreatic secretory trypsin inhibitor [Homo sapiens] >gi|190694 PSTI [Homo sapiens] >pir|A27484|TIHUA pancreatic secretory trypsin inhibitor precursor - human >sp|P00995|IPST_HUMAN PANCREATIC SECRETORY TRYPSIN INHIBITOR PRECURSOR (TUMOR-ASSOCIATED TRYPSI	gi|190688	194	475	86	86	HMQBB05
293	831255	MLN 64 [Homo sapiens] >dbj||D38255_1 CAB1 [Homo sapiens] >pir|I38027|I38027 MLN 64 protein - human >sp|Q14849|Q14849 MLN64 MRNA. Length = 445	gi|951279	198	431	85	92	HSDU60
294	831327	reg gene homologue [Homo sapiens] >gnl|PID|d1004610 regenerating protein I beta [Homo sapiens] >gnl|PID|d1004643 regenerating protein I beta [Homo sapiens] >pir|S34591|RGHU1B regenerating islet lectin 1-beta precursor - human >sp|P48304|LITB_HUMAN LITHOST	gi|487726	80	601	90	90	HPASG51
295	831493	DARPP-32=DOPAMINE AND CAMP-REGULATED PHOSPHOPROTEIN. >gi|1244402 DARPP-32 [Mus musculus] {SUB 1-27} Length = 204	sp|G545790|G545790	2	256	97	97	HSDF31

296	831500	endothelin 3 precursor [Homo sapiens] >pir|A34378|A34378 endothelin 3 precursor - human >sp|P14138|ET3_HUMAN ENDOTHELIN-3 PRECURSOR (ET-3). Length = 238	gi|182249	2	364	87	87	HWMEM06
297	831501			2	118			HSBO94
298	831502			339	527			HSCH48
299	831508			160	354			HCRMN21
300	831509			450	752			HSCC33
301	831520			242	424			HHTLE36
302	831547	match: protein P30711 [Homo sapiens] Length = 240	gnl|PID|e313869	2	766	91	92	HTYTA02
303	831548	glutathione transferase T1 [Homo sapiens] >pir|S44358|S44358 glutathione S-transferase Theta - human >sp|P30711|GTT1_HUMAN GLUTATHIONE S-TRANSFERASE THETA 1 (EC 2.5.1.18) (CLASS-THETA). {SUB 2-240} Length = 240	gi|510905	3	257	97	97	HHGDQ55
304	831558			3	410			HHGCU20
305	831847			48	953			HTPEI64
306	831893	(AF012023) integrin cytoplasmic domain associated protein; Icap-1a [Homo sapiens] >sp|O14713|O14713 INTEGRIN CYTOPLASMIC DOMAIN ASSOCIATED PROTEIN. Length = 200	gi|2305238	167	589	78	78	HPJDB54
307	831903			881	1045			HDTEA17

308	831921	(AF013965) Zis [Rattus norvegicus] >gi|2317754 (AF013966) Zis [Rattus norvegicus] >gi|2317756 (AF013967) Zis [Rattus norvegicus] >sp|O35986|O35986 ZIS. Length = 332	gi|2317752	66	410	70	78	HMUAR39
309	831923	(AF035527) EHF [Mus musculus] >sp|O70273|O70273 ETS HOMOLOGOUS FACTOR (EHF) (EHF). Length = 300	gi|3138930	133	1041	88	93	HDQEG93
310	831959			672	956			HDPRY54
311	832008	cyclin-dependent kinase [Homo sapiens] >pir|I68674|I68674 cyclin-dependent kinase - human (fragment) >gi|425143 cyclin-dependent kinase inhibitor [Homo sapiens] {SUB 18-181} Length = 181	gi|986879	1	741	86	86	HDPQA36
312	832107			2	244			HJACF80
313	832110	T-cell receptor alpha enhancer-binding protein, long form - human Length = 399	pir|A39625|A39625	2	844	91	91	HCFLR04
314	832146	CTP synthetase homolog [Mus musculus] >sp|P70303|P70303 CTP SYNTHETASE HOMOLOG (CTPSH). Length = 586	gi|1654186	3	587	69	77	HE8OJ09
315	832189			251	520			HISER65
316	832295	thymopoietin alpha [Homo sapiens] >pir|A55741|A55741 thymopoietin alpha precursor - human Length = 694	gi|508725	3	617	97	97	HCUDS28

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<tr><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th><th></th></tr>
<tr><td>317</td><td>832334</td><td>coatomer [Bos taurus] >pir|A49465|A49465 coatomer zeta chain - bovine >sp|P35604|COPZ_BOVIN COATOMER ZETA SUBUNIT (ZETA-COAT PROTEIN) (ZETA-COP). Length = 177</td><td>gi|441486</td><td>3</td><td>299</td><td>98</td><td>98</td><td>HBICG79</td></tr>
<tr><td>318</td><td>832339</td><td>(AF049105) centrosomal Nek2-associated protein 1 [Homo sapiens] >sp|O60588|O60588 CENTROSOMAL NEK2-ASSOCIATED PROTEIN 1. Length = 2442</td><td>gi|2984657</td><td>2</td><td>634</td><td>18</td><td>50</td><td>HE7TF56</td></tr>
<tr><td>319</td><td>832393</td><td>platelet-endothelial tetraspan antigen 3 [Homo sapiens] >sp|P48509|C151_HUMAN PLATELET-ENDOTHELIAL TETRASPAN ANTIGEN 3 (PETA-3) (GP27) (MEMBRANE GLYCOPROTEIN SFA-1) (CD151 ANTIGEN). Length = 253</td><td>gi|541613</td><td>49</td><td>591</td><td>81</td><td>81</td><td>HWLHD38</td></tr>
<tr><td>320</td><td>832415</td><td>PC4 [Homo sapiens] >gi|619161 PC4, p15 [Homo sapiens] >pir|A54670|A54670 RNA polymerase II transcription cofactor p15 - human >sp|P53999|P15_HUMAN ACTIVATED RNA POLYMERASE II TRANSCRIPTIONAL COACTIVATOR P15 (PC4) (P14). {SUB 2-127} Length = 127</td><td>gi|531395</td><td>80</td><td>475</td><td>87</td><td>87</td><td>HCDMB85</td></tr>
<tr><td>321</td><td>832422</td><td></td><td></td><td>3</td><td>1112</td><td></td><td></td><td>HAMGD22</td></tr>
<tr><td>322</td><td>832448</td><td>(AF006751) ES/130 [Homo sapiens] >sp|O75300|O75300 ES/130. Length = 977</td><td>gi|3299885</td><td>1</td><td>777</td><td>65</td><td>65</td><td>HAJBU71</td></tr>
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323	832532	protein serine/threonine kinase [Homo sapiens] >pir|A48082|A48082 mitogen-activated protein kinase p44-erk1 - human Length = 379	gi|31221	2	532	100	100	HMKDZ23
324	832621			1	462			H2CBH76
325	832622	(AF056209) PAM COOH-terminal interactor protein 1 [Homo sapiens] >gi|3560563 (AF056209) PAM COOH-terminal interactor protein 1 [Homo sapiens] >sp|O75901|O75901 PAM COOH-TERMINAL INTERACTOR PROTEIN 1. Length = 435	gi|3560563	78	569	97	97	H2CAA56
326	835327			140	355			HTPCS09
327	835695	(AF031174) Ig-like membrane protein [Homo sapiens] Length = 1215	gi|3766136	2	997	52	75	HDPFQ22
328	835857	(AC004549) TXBP151 [Homo sapiens] >sp|O60398|O60398 TXBP151. Length = 563	gi|3046307	1	1728	100	100	HEQBZ28
329	836183			46	978			HWLGV14
330	836190			1586	1813			HLTAV24
331	836196			2	319			HOECJ56
332	836253			1	363			HNSAC43
333	836372	Similar to sulfatase [Caenorhabditis elegans] >sp|Q21376|Q21376 SIMILAR TO SULFATASE. NCBI GI: 1125842. Length = 709	gi|1125842	794	1177	58	73	HOELR57
334	837077	similar to BPTI/KUNITZ inhibitor domain:	gnl|PID|e1345870	3	572	48	61	HEOMV66

335	837445	(AF046888) proliferation inducing ligand APRIL [Homo sapiens] >sp|O75888|O75888 PROLIFERATION INDUCING LIGAND APRIL. Length = 250	gi|3650492	801	1580	90	90	HDQFH76
336	837620	(AF002210) copper chaperone for superoxide dismutase [Homo sapiens] >sp|O14618|O14618 COPPER CHAPERONE FOR SUPEROXIDE DISMUTASE. Length = 274	gi|2431868	1	930	99	99	HTLDR72
337	837981	beta-1,6-N-acetylglucosaminyltransferase [Homo sapiens] >gi|886273 beta-1,6-N-acetylglucosaminyltransferase [Homo sapiens] >pir|A46293|A46293 beta-1,3-galactosyl-O-glycosyl-glycoprotein beta-1, 6-N-acetylglucosaminyltransferase (EC 2.4.1.102) - human >sp|	gi|183441	346	1740	58	74	HTPBB03
338	837995	aminopeptidase N precursor (EC 3.4.11.2) [Homo sapiens] >pir|A30325|A30325 membrane alanyl aminopeptidase (EC 3.4.11.2) precursor - human Length = 967	gi|178536	301	3231	94	94	HDPAY72

339	838001	lysyl hydroxylase isoform 2 [Homo sapiens] >sp|O00469|PLO2_HUMAN PROCOLLAGEN-LYSINE,2-OXOGLUTARATE 5-DIOXYGENASE 2 PRECURSOR (EC 1.14.11.4) (LYSYL HYDROXYLASE 2) (LH2). Length = 737	gi|2138314	1	2553	93	93	HOHAU14
340	838237	alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase [Gallus gallus] >pir|A49880|A49880 alpha-N-acetylgalactosaminide alpha-2,6-sialyltransferase (EC 2.4.99.3) - chicken >sp|Q92183|CAG3_CHICK ALPHA-N-ACETYLGALACTOSAMINIDE ALPHA-2,6-SIALYLTRANSFERASE	gi|453197	3	416	58	74	HWMFG72
341	838700			2	1756			HDTGB81
342	838805	(AL032653) similar to Ubiquitin-conjugating enzymes;	gnl|PID|e1350657	3	485	61	78	HAMGK18
343	839096	(AF027302) TNF-alpha stimulated ABC protein [Homo sapiens] >sp|O14897|O14897 TNF-ALPHA STIMULATED ABC PROTEIN. Length = 807	gi|2522534	666	1559	84	84	HDPJC76
344	839185	similar to ATP-binding transport protein family (ABC transporters) [Caenorhabditis elegans] >sp|Q20306|Q20306 GCN20 PROTEIN HOMOLOG. Length = 712	gi|500734	1323	2165	49	69	HDPFR49
345	839588			710	904			HLHEV35

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<tr><td>346</td><td>839589</td><td>synthetic preproinsulin [artificial sequence] >gi|58103 reading frame proinsulin [unidentified] {SUB 28-114} >gi|208664 insulin B chain [artificial sequence] {SUB 28-58} >gi|208660 insulin beta chain [artificial sequence] {SUB 29-58} >gi|929915 insulin C</td><td>gi|208668</td><td>1</td><td>498</td><td>80</td><td>80</td><td>HCCMB04</td></tr>
<tr><td>347</td><td>839733</td><td>RGL2 [Homo sapiens] >sp|O15211|O15211 RGL2. >gnl|PID|d1037179 (AB012295) GDS-related protein [Homo sapiens] {SUB 656-777} Length = 777</td><td>gnl|PID|e1186796</td><td>1098</td><td>2681</td><td>90</td><td>90</td><td>HYACI02</td></tr>
<tr><td>348</td><td>839874</td><td>mitochondrial NAD(P)+ -dependent malic enzyme [Homo sapiens] >pir|A39503|A39503 malate dehydrogenase (NAD+) (EC 1.1.1.-) precursor, mitochondrial - human >sp|P23368|MAOM_HUMAN MALATE OXIDOREDUCTASE [NAD], MITOCHONDRIAL PRECURSOR (EC 1.1.1.40) (MALIC ENZYM</td><td>gi|187300</td><td>102</td><td>1058</td><td>96</td><td>97</td><td>HDPPO41</td></tr>
<tr><td>349</td><td>840017</td><td>FKBP65 binding protein [Mus musculus] >pir|I49669|I49669 FKBP65 binding protein - mouse >sp|Q61576|Q61576 FK506 BINDING PROTEIN 6 (65 KDA) (FKBP65 BINDING PROTEIN). Length = 581</td><td>gi|894162</td><td>2</td><td>1303</td><td>86</td><td>92</td><td>HLHBI67</td></tr>
<tr><td>350</td><td>840124</td><td></td><td></td><td>937</td><td>1311</td><td></td><td></td><td>HNHGX94</td></tr>
<tr><td>351</td><td>840222</td><td>P24 protein [Mus musculus] >sp|P97799|P97799 VESICULAR MEMBRAIN PROTEIN P24 (P24 PROTEIN). Length = 196</td><td>gnl|PID|d1019688</td><td>28</td><td>747</td><td>35</td><td>47</td><td>HSES36</td></tr>
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352	840617	(AF033861) type III adenylyl cyclase [Homo sapiens] >sp|G4104226|G4104226 TYPE III ADENYLYL CYCLASE. >gnl|PID|d1026367 (AB011083) KIAA0511 protein [Homo sapiens] {SUB 212-1144} >sp|G299652|G299652 TYPE III ADENYLYL CYCLASE. TYPE III AC {EC 4.6.1.1}. {SUB	gi|4104226	35	2599	91	91	HTPDM11
353	840641	RGS10 protein - human Length = 173	pir|S71812|S71812	2	622	100	100	HULFJ24
354	840792	sigma 3A protein [Homo sapiens] >gi|1923270 AP-3 complex sigma3A subunit [Homo sapiens] >gnl|PID|d1010444 clathrin coat assembly protein-like [Homo sapiens] >gi|3462900 (AF084575) adaptor protein complex-3 sigma3A subunit isoform [Mus musculus] >gi|192327	gnl|PID|e256812	1	699	100	100	HOEJT19
355	840915	Tob [Homo sapiens] Length = 345	gnl|PID|d1008002	411	1469	82	82	H2MBF94
356	841059			583	828			HSBL50
357	841325	FAC1 gene product [Homo sapiens] >pir|G01252|G01252 small GTP binding protein, homologous to SEC4 - human >sp|Q12830|Q12830 FETAL ALZ-50-REACTIVE CLONE 1 (FAC1). Length = 810	gi|1276428	6	989	95	95	HFEBE52
358	841713			3	872			HWHGW75
359	842324			487	837			HLWBQ31
360	842386			201	671			HCFDA62

361	842454	mitochondrial ATPase inhibitor [Rattus norvegicus] >gnl|PID|d1002924 ATPase inhibitor protein precursor [Rattus sp.] >pir|JS0738|JS0738 ATPase inhibitor protein precursor, mitochondrial - rat >sp|Q03344|IATP_RAT ATPASE INHIBITOR, MITOCHONDRIAL PRECURSOR.	gi|517226	1	231	76	88	HVAMF27
362	842768			179	400			HRABQ15
363	842999			2	1354			HNTSM88
364	843830	dJ434P1.3 [Homo sapiens] >gi|1592565 DEAD-box protein p72 [Homo sapiens] >pir|S72367|S72367 ATP-dependent RNA helicase - human >sp|Q92841|P72_HUMAN PROBABLE RNA-DEPENDENT HELICASE P72 (DEAD-BOX PROTEIN P72). Length = 650	gnl|PID|e1249592	3	635	93	93	HMSPB89
365	844723			1	66			HLYBO68
366	844868	(AF092557) LIM domain only 7 [Homo sapiens] >sp|G4028544|G4028544 LIM DOMAIN ONLY 7 (FRAGMENT). Length = 120	gi|4028544	1	1179	56	74	HHPBC57
367	845258	MRAS2 gene product [Rhizomucor racemosus] Length = 198	gi|553070	131	925	30	45	HKAKW86
368	845373			183	1847			HNTEE61
369	845412	(AB003184) ISLR [Homo sapiens] >sp|O14498|O14498 ISLR PRECURSOR. Length = 428	dbj||AB003184_1	303	1178	87	87	HCRNP15
370	HISED43R			29	283			HISED43

371	HOSEQ76R			165	308			HOSEQ76
372	HSDS43R			1	141			HSDS43
373	HPJDY28R			53	136			HPJDY28
374	HTPBW71R			29	94			HTPBW71
375	HCQAG14R			18	173			HCQAG14
376	HVANP48R			14	271			HVANP48
377	HGBGO86R	(AB005546) porcine serum amyloid P component (SAP) [Sus scrofa] >sp|O19063|O19063 PORCINE SERUM AMYLOID P COMPONENT (SAP) PRECURSOR (SAP). Length = 224	gnl|PID|d1022321	2	139	66	76	HGBGO86
378	HSDW59R	(AB012223) ORF2 [Canis familiaris] >sp|O62658|O62658 LINE-1 ELEMENT ORF2. Length = 1275	gnl|PID|d1026181	261	647	51	72	HSDW59
379	HDBAM16R	(AF000381) non-functional folate binding protein [Homo sapiens] >sp|O14597|O14597 NON-FUNCTIONAL FOLATE BINDING PROTEIN. Length = 254	gi|2565196	189	416	88	88	HDBAM16
380	HTPGD92R	(AF016692) small intestinal mucin MUC3 [Homo sapiens] >pir|PC4395|PC4395 mucin 3 - human (fragment) >sp|O14760|O14760 SMALL INTESTINAL MUCIN MUC3 (FRAGMENT). Length = 648	gi|2454615	3	308	46	46	HTPGD92

381	HHFLB69R	(AF018432) dUTPase [Homo sapiens] >gi|1144332 deoxyuridine nucleotidohydrolase [Homo sapiens] >gi|1421818 deoxyuridine triphosphatase [Homo sapiens] >pir|G02777|G02777 dUTP pyrophosphatase (EC 3.6.1.23) - human >gi|292877 dUTP nucleotidohydrolase [Homo sa	gi|2443581	3	245	100	100	HHFLB69
382	HPDEH50R	(AF026689) prostate-specific transglutaminase [Homo sapiens] >sp|O75320|O75320 PROSTATE-SPECIFIC TRANSGLUTAMINASE (FRAGMENT). Length = 51	gi|3523113	2	166	66	72	HPDEH50
383	HMTMA16R	(AF042081) SH3 domain binding glutamic acid-rich-like protein [Homo sapiens] >sp|O75368|O75368 SH3 DOMAIN BINDING GLUTAMIC ACID-RICH-LIKE PROTEIN. Length = 114	gi|3337420	3	299	100	100	HMTMA16
384	HTNAP71R	(AF080484) thyroglobulin [Homo sapiens] >sp|G3415051|G3415051 THYROGLOBULIN (FRAGMENT). Length = 680	gi|3415051	2	199	95	95	HTNAP71
385	HTPGL88R	(AF081673) bile salt-dependent lipase oncofetal isoform [Homo sapiens] >sp|O75612|O75612 BILE SALT-DEPENDENT LIPASE ONCOFETAL ISOFORM (FRAGMENT). Length = 612	gi|3421403	3	434	97	98	HTPGL88

386	HMCIA86R	actin [Absidia glauca] >pir|S03109|S03109 actin - pin mould (Absidia glauca) (fragment) >sp|P10982|ACT1_ABSGL ACTIN 1 (FRAGMENT). >gi|669036 actin [Absidia glauca] {SUB 3-140} Length = 140	gi|578097	2	250	88	100	HMCIA86
387	HAPOC60R	alpha-catenin [Homo sapiens] >gi|4092761 (AF102803) alphaE-catenin [Homo sapiens] >pir|JN0607|JN0607 alpha-catenin - human >sp|P35221|CTN1_HUMAN ALPHA-1 CATENIN (CADHERIN-ASSOCIATED PROTEIN) (ALPHA E-CATENIN). Length = 906	gnl|PID|d1003485	2	505	77	80	HAPOC60
388	HDTFE89R	antibody, heavy chain variable regin to HIV1 gp120 [Homo sapiens] Length = 127	gi|732750	3	329	75	78	HDTFE89
389	HAJBO38R	Bat2 [Homo sapiens] >pir|S37671|S37671 bat2 protein - human Length = 1870	gi|29375	1	435	94	94	HAJBO38
390	HCCMA90R	BILE SALT-DEPENDENT LIPASE. Length = 720	sp|Q16398|Q16398	3	329	75	75	HCCMA90
391	HTLHH34R	carnitine O-acetyltransferase (EC 2.3.1.7) precursor, mitochondrial - human >sp|P43155|CACP_HUMAN CARNITINE O-ACETYLTRANSFERASE (EC 2.3.1.7) (CARNITINE ACETYLASE) (CAT) (FRAGMENT). {SUB 3-626} Length = 626	pir|A55720|A55720	3	602	97	97	HTLHH34

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<tr><td>392</td><td>HWHPY22R</td><td>CLN3 protein [Homo sapiens] >gnl|PID|e283670 CLN3 protein [Homo sapiens] >gi|2947055 (AC002425) CLN3 [Homo sapiens] >gi|3337387 (AC002544) CLN [Homo sapiens] >gi|4102729 (AF015593) CLN3 protein [Homo sapiens] >pir|A57219|A57219 Batten disease-related prot</td><td>gi|1039423</td><td>1</td><td>432</td><td>95</td><td>95</td><td>HWHPY22</td></tr>
<tr><td>393</td><td>HCWFF39R</td><td>collagen alpha 1(V) chain precursor [Homo sapiens] >sp|P20908|CA15_HUMAN PROCOLLAGEN ALPHA 1(V) CHAIN PRECURSOR. >gi|1020326 alpha-1 type V collagen [Homo sapiens] {SUB 1-36} Length = 1838</td><td>gnl|PID|d1015029</td><td>3</td><td>407</td><td>65</td><td>65</td><td>HCWFF39</td></tr>
<tr><td>394</td><td>HBBBA92R</td><td>cytochrome oxidase subunit I [Anas platyrhynchos] >sp|P50656|COX1_ANAPL CYTOCHROME C OXIDASE POLYPEPTIDE I (EC 1.9.3.1) (FRAGMENT). Length = 102</td><td>gi|348683</td><td>31</td><td>240</td><td>41</td><td>58</td><td>HBBBA92</td></tr>
<tr><td>395</td><td>HTLHP03R</td><td>dipeptidase precursor [Homo sapiens] Length = 411</td><td>gnl|PID|d1002931</td><td>3</td><td>413</td><td>92</td><td>92</td><td>HTLHP03</td></tr>
<tr><td>396</td><td>HCCMA63R</td><td>elastase III B [Homo sapiens] >pir|B29934|B29934 pancreatic elastase (EC 3.4.21.36) IIIB precursor - human >sp|P08861|EL3B_HUMAN ELASTASE IIIB PRECURSOR (EC 3.4.21.70) (PROTEASE E). Length = 270</td><td>gi|182035</td><td>1</td><td>252</td><td>96</td><td>96</td><td>HCCMA63</td></tr>
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397	HE8EZ78R	endosomal protein [Homo sapiens] >pir|S44243|S44243 endosomal protein - human >sp|Q15075|Q15075 ENDOSOMAL PROTEIN. Length = 1411	gi|475934	2	352	98	100	HE8EZ78
398	HGLAQ29R	erythroid DNA-binding protein [Homo sapiens] >gi|31243 Eryf1 transcription factor (AA 1-413) [Homo sapiens] >pir|A34888|A34888 transcription factor GATA-1 - human >sp|P15976|GAT1_HUMAN ERYTHROID TRANSCRIPTION FACTOR (GATA-1) (ERYF1) (GF-1) (NF-E1). Length	gi|183072	1	186	50	54	HGLAQ29
399	HALSD82R	fibrinogen gamma-prime chain [Homo sapiens] >sp|P04469|FIBH_HUMAN FIBRINOGEN GAMMA-B CHAIN PRECURSOR (FIBRINOGEN GAMMA'). >gi|182443 gamma fibrinogen type B (AA at 202) [Homo sapiens] {SUB 285-453} Length = 453	gi|182440	1	399	98	100	HALSD82
400	H2LAS44R	gamma subunit of CCT chaperonin [Homo sapiens] >pir|S61529|A38983 TCP1 ring complex protein TRiC5 - human Length = 544	gi|671527	75	560	99	99	H2LAS44
401	HTXPA42R	GTP-binding protein (rab7) [Canis familiaris] >pir|B30413|B30413 GTP-binding protein rab7 - dog Length = 207	gi|164058	166	432	98	100	HTXPA42

<table>
<tr><td>402</td><td>HBWAH57R</td><td>hCRMP-2 [Homo sapiens] >gnl|PID|d1011853 dihydropyrimidinase related protein-2 [Homo sapiens] >gi|2967519 N2A3 [Homo sapiens] >pir|JC5317|JC5317 dihydropyrimidinase-related protein 2 - human >sp|Q16555|DPY2_HUMAN DIHYDROPYRIMIDINASE RELATED PROTEIN-2 (DRP</td><td>gi|1244400</td><td>2</td><td>121</td><td>92</td><td>94</td><td>HBWAH57</td></tr>
<tr><td>403</td><td>HAHEJ39R</td><td>HSJ1a [Homo sapiens] >pir|S23509|S23509 dnaJ protein homolog - human Length = 277</td><td>gi|32470</td><td>2</td><td>370</td><td>84</td><td>84</td><td>HAHEJ39</td></tr>
<tr><td>404</td><td>HOEMQ04R</td><td>hypoxia-inducible factor 1 alpha [Homo sapiens] >gi|1144013 ARNT interacting protein [Homo sapiens] >pir|138972|138972 hypoxia-inducible factor 1 alpha - human >sp|Q16665|HIFA_HUMAN HYPOXIA-INDUCIBLE FACTOR 1 ALPHA (HIF-1 ALPHA) (ARNT INTERACTING PROTEIN)</td><td>gi|881346</td><td>3</td><td>299</td><td>100</td><td>100</td><td>HOEMQ04</td></tr>
<tr><td>405</td><td>HAPBR18R</td><td>Ig kappa L-chain variable region [Homo sapiens] Length = 122</td><td>gi|1905938</td><td>29</td><td>325</td><td>67</td><td>70</td><td>HAPBR18</td></tr>
<tr><td>406</td><td>HOENU56R</td><td></td><td></td><td>1</td><td>288</td><td></td><td></td><td>HOENU56</td></tr>
<tr><td>407</td><td>HAGGB37R</td><td>L-arginine: glycine amidinotransferase [Homo sapiens] >pir|S54161|S54161 L-arginine--glycine amidinotransferase - human Length = 391</td><td>gi|791049</td><td>2</td><td>238</td><td>83</td><td>83</td><td>HAGGB37</td></tr>
</table>

408	HCCMC02R	lipase related protein 2 [Homo sapiens] >pir|B43357|B43357 pancreatic lipase-related protein 2 - human >sp|P54317|LIP2_HUMAN PANCREATIC LIPASE RELATED PROTEIN 2 PRECURSOR (EC 3.1.1.3). Length = 469	gi|187232	1	357	61	64	HCCMC02
409	HAHDO57R	located at OATL1 [Homo sapiens] >sp|Q14827|Q14827 DNA SEGMENT, JOHNS HOPKINS UNIVERSITY 1 (MG21) (FRAGMENT). Length = 166	gi|950411	3	536	100	100	HAHDO57
410	HOEMK29R	lysyl oxidase-2 [Mus musculus] >sp|P97873|P97873 LYSYL OXIDASE-LIKE (LYSYL OXIDASE-2) (LYSYL OXIDASE-LIKE PROTEIN) (FRAGMENT). Length = 110	gi|2636697	3	116	97	97	HOEMK29
411	HRADJ65R	ORF protein; C-terminal (aa 125-319; 196aa) [Homo sapiens] Length = 196	gi|37610	1	357	98	98	HRADJ65
412	HTPCT95R	pancreatic elastase IIB zymogen [Homo sapiens] >pir|C26823|C26823 pancreatic elastase II (EC 3.4.21.71) B precursor - human >sp|P08218|EL2B_HUMAN ELASTASE 2B PRECURSOR (EC 3.4.21.71). Length = 269	gi|182060	2	340	98	98	HTPCT95
413	HLQFY56R	pancreatitis associated protein [Homo sapiens] Length = 174	gi|189601	281	493	94	94	HLQFY56

414	HCCMD33R	phospholipase [Homo sapiens] >gi|387025 phospholipase [Homo sapiens] >gi|2769697 (AC003982) Phosphatidylcholine 2-acylhydrolase [Homo sapiens] >pir|C25793|PSHU phospholipase A2 (EC 3.1.1.4) precursor, pancreatic - human >sp|P04054|PA21_HUMAN PHOSPHOLIPASE	gi|190013	109	345	77	77	HCCMD33
415	HDPAQ04R	PQ-rich protein [Homo sapiens] >pir|S58222|S58222 PQ-rich protein - human >sp|Q15184|Q15184 PQ-RICH PROTEIN. Length = 400	gi|929660	3	125	85	85	HDPAQ04
416	HCE4L96R	PRSM1 [Homo sapiens] >pir|JC4963|JC4963 metalloproteinase 1 (EC 3.4.24.-) - human >sp|Q15779|Q15779 PRSM1. Length = 318	gi|1354931	3	194	89	89	HCE4L96
417	HTPGL86R	putative surface glycoprotein [Homo sapiens] >sp|P53801|C211_HUMAN PUTATIVE SURFACE GLYCOPROTEIN C21ORF1 PRECURSOR (C21ORF3). Length = 180	gnl|PID|e188111	32	322	85	85	HTPGL86

418	HLQGB61R	reg gene homologue [Homo sapiens] >gnl|PID|d1004610 regenerating protein I beta [Homo sapiens] >gnl|PID|d1004643 regenerating protein I beta [Homo sapiens] >pir|S34591|RGHU1B regenerating islet lectin 1-beta precursor - human >sp|P48304|LITB_HUMAN LITHOST	gi|487726	21	182	92	92	HLQGB61
419	HWDAK95R	RNA splicing-related protein [Rattus norvegicus] >sp|O54729|O54729 BRAIN. Length = 712	gnl|PID|d1024790	141	362	82	88	HWDAK95
420	HE9DG72R	selenium-binding protein [Homo sapiens] >pir|G01872|G01872 selenium-binding protein - human >sp|Q13228|Q13228 SELENIUM-BINDING PROTEIN. Length = 472	gi|1374792	1	414	95	96	HE9DG72
421	HDPOY89R	Similar to sulfatase [Caenorhabditis elegans] >sp|Q21376|Q21376 SIMILAR TO SULFATASE. NCBI GI: 1125842. Length = 709	gi|1125842	132	452	47	71	HDPOY89
422	HAHEJ13R	sperm membrane protein [Rattus norvegicus] >pir|A35981|A35981 sperm membrane protein - rat Length = 191	gi|207694	1	366	58	60	HAHEJ13
423	HOEMR16R	tyrosine phosphatase precursor [Homo sapiens] >sp|Q14513|Q14513 TYROSINE PHOSPHATASE PRECURSOR (EC 3.1.3.48). Length = 793	gi|32067	3	80	76	84	HOEMR16
424	HCFCM83R	ubiquitin--protein ligase E1 homolog - human Length = 1058	pir|A48195|A48195	168	269	94	94	HCFCM83
425	H6BSB07R			1	105			H6BSB07

426	HAGCC01R	1	219	HAGCC01
427	HAQAM88R	49	372	HAQAM88
428	HAUBA62R	3	233	HAUBA62
429	HBCMA07R	3	164	HBCMA07
430	HBGNU45R	132	293	HBGNU45
431	HBJHW09R	142	309	HBJHW09
432	HBMBJ92R	3	173	HBMBJ92
433	HCGBC37R	1	219	HCGBC37
434	HCROI22R	105	236	HCROI22
435	HDTLK21R	184	351	HDTLK21
436	HDTLX11R	234	518	HDTLX11
437	HE2CM25R	27	470	HE2CM25
438	HE9FI19R	1	183	HE9FI19
439	HEGAD29R	234	344	HEGAD29
440	HFKHC10R	118	267	HFKHC10
441	HFPAE25R	174	332	HFPAE25
442	HGBHA95R	3	161	HGBHA95
443	HHBEA82R	139	387	HHBEA82
444	HISCX64R	1	312	HISCX64
445	HLCAB30R	1	72	HLCAB30
446	HLDOW24R	147	296	HLDOW24
447	HLLBA89R	56	220	HLLBA89
448	HLQDE48R	299	523	HLQDE48
449	HNEDI54R	1	84	HNEDI54
450	HNHGQ70R	1	423	HNHGQ70
451	HOSMV19R	151	291	HOSMV19
452	HTPGJ41R	89	340	HTPGJ41
453	HTTHJ11R	1	90	HTTHJ11
454	HULEB88R	2	376	HULEB88
455	HUSJN92R	284	460	HUSJN92
456	HWAEJ52R	1	285	HWAEJ52
457	HWLMS12R	136	321	HWLMS12

458	HWLWG58R			1	108			HWLWG58
459	HAIDL46R	X104 [Homo sapiens] >pir|I54378|I54378 gene X104 protein - human >sp|Q15883|Q15883 X104. >gi|3462868 (AF083892) tight junction protein ZO-2 isoform A [Homo sapiens] {SUB 1-166} >gi|3462870 (AF083893) tight junction protein ZO-2 isoform C [Homo sapiens] {S	gi|498013	3	224	55	57	HAIDL46

The first column of Table 1 shows the "SEQ ID NO:" for each of the 459 pancreatic cancer antigen polynucleotide sequences of the invention.

The second column in Table 1, provides a unique "Sequence/Contig ID" identification for each pancreas and/or pancreatic cancer associated sequence. The third column in Table 1, "Gene Name," provides a putative identification of the gene based on the sequence similarity of its translation product to an amino acid sequence found in a publicly accessible gene database, such as GenBank (NCBI). The great majority of the cDNA sequences reported in Table 1 are unrelated to any sequences previously described in the literature. The fourth column, in Table 1, "Overlap," provides the database accession no. for the database sequence having similarity. The fifth and sixth columns in Table 1 provide the location (nucleotide position nos. within the contig), "Start" and "End", in the polynucleotide sequence "SEQ ID NO:X" that delineate the preferred ORF shown in the sequence listing as SEQ ID NO:Y. In one embodiment, the invention provides a protein comprising, or alternatively consisting of, a polypeptide encoded by the portion of SEQ ID NO:X delineated by the nucleotide position nos. "Start" and "End". Also provided are polynucleotides encoding such proteins and the complementary strand thereto. The seventh and eighth columns provide the "% Identity" (percent identity) and "% Similarity" (percent similarity) observed between the aligned sequence segments of the translation product of SEQ ID NO:X and the database sequence.

The ninth column of Table 1 provides a unique "Clone ID" for a clone related to each contig sequence. This clone ID references the cDNA clone which contains at least the 5' most sequence of the assembled contig and at least a portion of SEQ ID NO:X was determined by directly sequencing the referenced clone. The reference clone may have more sequence than described in the sequence listing or the clone may have less. In the vast majority of cases, however, the clone is believed to encode a full-length polypeptide. In the case where a clone is not full-length, a full-length cDNA can be obtained by methods described elsewhere herein.

Table 3 indicates public ESTs, of which at least one, two, three, four, five, ten, or more of any one or more of these public ESTs are optionally excluded from the invention.

SEQ ID NO:X (where X may be any of the polynucleotide sequences disclosed in the sequence listing as SEQ ID NO:1 through SEQ ID NO:459) and the translated SEQ ID NO:Y (where Y may be any of the polypeptide sequences disclosed in the sequence listing as SEQ ID NO:460 through SEQ ID NO:918) are sufficiently accurate and otherwise suitable for a

variety of uses well known in the art and decribed further below. For instance, SEQ ID NO:X has uses including, but not limited to, in designing nucleic acid hybridization probes that will detect nucleic acid sequences contained in SEQ ID NO:X or the related cDNA clone contained in a library deposited with the ATCC. These probes will also hybridize to nucleic acid molecules in biological samples, thereby enabling immediate applications in chromosome mapping, linkage analysis, tissue identification and/or typing, and a variety of forensic and diagnostic methods of the invention. Similarly, polypeptides identified from SEQ ID NO:Y have uses that include, but are not limited to, generating antibodies which bind specifically to the pancreatic cancer antigen polypeptides, or fragments thereof, and/or to the pancreatic cancer antigen polypeptides encoded by the cDNA clones identified in Table 1.

Nevertheless, DNA sequences generated by sequencing reactions can contain sequencing errors. The errors exist as misidentified nucleotides, or as insertions or deletions of nucleotides in the generated DNA sequence. The erroneously inserted or deleted nucleotides cause frame shifts in the reading frames of the predicted amino acid sequence. In these cases, the predicted amino acid sequence diverges from the actual amino acid sequence, even though the generated DNA sequence may be greater than 99.9% identical to the actual DNA sequence (for example, one base insertion or deletion in an open reading frame of over 1000 bases).

Accordingly, for those applications requiring precision in the nucleotide sequence or the amino acid sequence, the present invention provides not only the generated nucleotide sequence identified as SEQ ID NO:X, the predicted translated amino acid sequence identified as SEQ ID NO:Y, but also a sample of plasmid DNA containing the related cDNA clone (deposited with the ATCC, as set forth in Table 1). The nucleotide sequence of each deposited clone can readily be determined by sequencing the deposited clone in accordance with known methods. Further, techniques known in the art can be used to verify the nucleotide sequences of SEQ ID NO:X.

The predicted amino acid sequence can then be verified from such deposits. Moreover, the amino acid sequence of the protein encoded by a particular clone can also be directly determined by peptide sequencing or by expressing the protein in a suitable host cell containing the deposited human cDNA, collecting the protein, and determining its sequence.

The present invention also relates to vectors or plasmids which include such DNA sequences, as well as the use of the DNA sequences. The material deposited with the ATCC on:

Table 2

ATCC Deposits	Deposit Date	ATCC Designation Number
LP01, LP02, LP03, LP04, LP05, LP06, LP07, LP08, LP09, LP10, LP11,	May-20-97	209059, 209060, 209061, 209062, 209063, 209064, 209065, 209066, 209067, 209068, 209069
LP12	Jan-12-98	209579
LP13	Jan-12-98	209578
LP14	Jul-16-98	203067
LP15	Jul-16-98	203068
LP16	Feb-1-99	203609
LP17	Feb-1-99	203610
LP20	Nov-17-98	203485
LP21	Jun-18-99	PTA-252
LP22	Jun-18-99	PTA-253
LP23	Dec-22-99	PTA-1081

each is a mixture of cDNA clones derived from a variety of human tissue and cloned in either a plasmid vector or a phage vector, as shown in Table 5. These deposits are referred to as "the deposits" herein. The tissues from which the clones were derived are listed in Table 5, and the vector in which the cDNA is contained is also indicated in Table 5. The deposited material includes the cDNA clones which were partially sequenced and are related to the SEQ ID NO:X described in Table 1 (column 9). Thus, a clone which is isolatable from the ATCC Deposits by use of a sequence listed as SEQ ID NO:X may include the entire coding region of a human gene or in other cases such clone may include a substantial portion of the coding region of a human gene. Although the sequence listing lists only a portion of the DNA sequence in a clone included in the ATCC Deposits, it is well within the ability of one skilled in the art to complete the sequence of the DNA included in a clone isolatable from the

ATCC Deposits by use of a sequence (or portion thereof) listed in Table 1 by procedures hereinafter further described. and others apparent to those skilled in the art.

Also provided in Table 5 is the name of the vector which contains the cDNA clone. Each vector is routinely used in the art. The following additional information is provided for convenience.

Vectors Lambda Zap (U.S. Patent Nos. 5,128,256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5.286,636). pBluescript (pBS) (Short, J. M. et al., *Nucleic Acids Res. 16*:7583-7600 (1988); Alting-Mees, M. A. and Short. J. M., *Nucleic Acids Res. 17*:9494 (1989)) and pBK (Alting-Mees. M. A. et al., *Strategies 5*:58-61 (1992)) are commercially available from Stratagene Cloning Systems. Inc.. 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Phagemid pBS may be excised from the Lambda Zap and Uni-Zap XR vectors, and phagemid pBK may be excised from the Zap Express vector. Both phagemids may be transformed into *E. coli* strain XL-1 Blue, also available from Stratagene.

Vectors pSport1, pCMVSport 1.0, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, also available from Life Technologies. See, for instance, Gruber, C. E., et al., *Focus 15*:59 (1993). Vector lafmid BA (Bento Soares, Columbia University, New York, NY) contains an ampicillin resistance gene and can be transformed into *E. coli* strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into *E. coli* strain DH10B, available from Life Technologies. See, for instance, Clark, J. M., *Nuc. Acids Res. 16*:9677-9686 (1988) and Mead, D. *et al., Bio/Technology 9*: (1991).

The present invention also relates to the genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in a deposited cDNA clone. The corresponding gene can be isolated in accordance with known methods using the sequence information disclosed herein. Such methods include, but are not limited to, preparing probes or primers from the disclosed sequence and identifying or amplifying the corresponding gene from appropriate sources of genomic material.

Also provided in the present invention are allelic variants, orthologs, and/or species homologs. Procedures known in the art can be used to obtain full-length genes, allelic variants, splice variants, full-length coding portions, orthologs, and/or species homologs of genes corresponding to SEQ ID NO:X, SEQ ID NO:Y, and/or the cDNA contained in the related cDNA clone in the deposit, using information from the sequences disclosed herein or the clones deposited with the ATCC. For example, allelic variants and/or species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source for allelic variants and/or the desired homologue.

The present invention provides a polynucleotide comprising, or alternatively consisting of, the nucleic acid sequence of SEQ ID NO:X, and/or the related cDNA clone (See, e.g., columns 1 and 9 of Table 1). The present invention also provides a polypeptide comprising, or alternatively, consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library. Polynucleotides encoding a polypeptide comprising, or alternatively consisting of, the polypeptide sequence of SEQ ID NO:Y, a polypeptide encoded by SEQ ID NO:X, and/or a polypeptide encoded by the the dDNA in the related cDNA clone contained in a deposited library, are also encompassed by the invention. The present invention further encompasses a polynucleotide comprising, or alternatively consisting of, the complement of the nucleic acid sequence of SEQ ID NO:X, and/or the complement of the coding strand of the related cDNA clone contained in a deposited library.

Many polynucleotide sequences, such as EST sequences, are publicly available and accessible through sequence databases and may have been publicly available prior to conception of the present invention. Preferably, such related polynucleotides are specifically excluded from the scope of the present invention. To list every related sequence would unduly burden the disclosure of this application. Accordingly, for each "Contig Id" listed in the first column of Table 3, preferably excluded are one or more polynucleotides comprising a nucleotide sequence described in the second column of Table 3 by the general formula of a-b, each of which are uniquely defined for the SEQ ID NO:X corresponding to that Contig Id in Table 1. Additionally, specific embodiments are directed to polynucleotide sequences excluding at least one, two, three, four, five, ten, or more of the specific polynucleotide sequences referenced by the Genbank Accession No. for each Contig Id which may be

included in column 3 of Table 3. In no way is this listing meant to encompass all of the sequences which may be excluded by the general formula, it is just a representative example.

Table 3.

Sequence/ Contig ID	General formula	Genbank Accession No.
456379	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 551 of SEQ ID NO:1, b is an integer of 15 to 565, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:1, and where b is greater than or equal to a + 14.	R34554, AA018972, AA055489
462108	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1677 of SEQ ID NO:2, b is an integer of 15 to 1691, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:2, and where b is greater than or equal to a + 14.	T79903, R46289, R73001, R73606, N30140, N35752, W32520, W32636, AA018675, AA018676, AA040600, AA040683, AA070495, AA070381, AA083072, AA134451, AA207060, AA207086
503446	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 466 of SEQ ID NO:3, b is an integer of 15 to 480, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:3, and where b is greater than or equal to a + 14.	
507841	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 594 of SEQ ID NO:4, b is an integer of 15 to 608, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:4, and where b is greater than or equal to a + 14.	R12126, R14285
509287	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 682 of SEQ ID NO:5, b is an integer of 15 to 696, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:5, and where b is greater than or equal to a + 14.	H01699, H94037, N30572, N57219, N64393, N92189, AA035664, AA037022, AA045335, AA045422, AA056367, AA115587
509672	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 278 of SEQ ID NO:6, b is an integer of 15 to 292, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:6, and where b is greater than or equal to a + 14.	
509673	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 348 of SEQ ID NO:7, b is an integer of 15 to 362, where both a and	

	b correspond to the positions of nucleotide residues shown in SEQ ID NO:7. and where b is greater than or equal to a + 14.	
518767	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 391 of SEQ ID NO:8. b is an integer of 15 to 405, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:8. and where b is greater than or equal to a + 14.	
522008	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1013 of SEQ ID NO:9. b is an integer of 15 to 1027, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:9, and where b is greater than or equal to a + 14.	T63280. R50010. R78743. R78742. H52248. H52346. H91191. AA028894. AA031289. AA121197, AA150816, AA160833
524112	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1501 of SEQ ID NO:10, b is an integer of 15 to 1515. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:10, and where b is greater than or equal to a + 14.	H49520. H66748. H68803. H68904, N45520. W42600. W42573. AA134942, AA151361. AA227110. AA251434
525971	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 833 of SEQ ID NO:11. b is an integer of 15 to 847. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:11, and where b is greater than or equal to a + 14.	W81027. AA133066
527156	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 492 of SEQ ID NO:12. b is an integer of 15 to 506, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:12. and where b is greater than or equal to a + 14.	W23806
532502	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 253 of SEQ ID NO:13. b is an integer of 15 to 267. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:13. and where b is greater than or equal to a + 14.	
533459	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 905 of SEQ ID NO:14. b is an integer of 15 to 919, where both a and b correspond to the positions of nucleotide residues	

	shown in SEQ ID NO:14, and where b is greater than or equal to a + 14.	
533551	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2545 of SEQ ID NO:15, b is an integer of 15 to 2559, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:15, and where b is greater than or equal to a + 14.	H44763, H44764, AA011378, AA011366, AA215758
537850	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1490 of SEQ ID NO:16, b is an integer of 15 to 1504, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:16, and where b is greater than or equal to a + 14.	T68458, T68523, T83911, R07511, R07564, R10442, R11516, T80802, T81206, T83580, T83740, T85796, R06434, R06489, H40512, H47544, H47543, R85697, R89315, R89396, R91325, R96709, H59265, H59311, H64291, H78244, H78445, H90091, H94341, H94427
537925	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 819 of SEQ ID NO:17, b is an integer of 15 to 833, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:17, and where b is greater than or equal to a + 14.	AA085845
538160	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 629 of SEQ ID NO:18, b is an integer of 15 to 643, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:18, and where b is greater than or equal to a + 14.	W52418
540420	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 326 of SEQ ID NO:19, b is an integer of 15 to 340, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:19, and where b is greater than or equal to a + 14.	
540802	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 659 of SEQ ID NO:20, b is an integer of 15 to 673, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:20, and where b is greater than or equal to a + 14.	R27496, W05560, W40286, AA147911
540989	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 401 of SEQ ID NO:21, b is an integer of 15 to 415, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:21, and where b is greater than	

	or equal to a + 14.	
540997	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 619 of SEQ ID NO:22, b is an integer of 15 to 633, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:22, and where b is greater than or equal to a + 14.	W39752
548735	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2409 of SEQ ID NO:23, b is an integer of 15 to 2423, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:23, and where b is greater than or equal to a + 14.	T61438, R72243, AA134330
549709	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 370 of SEQ ID NO:24, b is an integer of 15 to 384, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:24, and where b is greater than or equal to a + 14.	
550007	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 886 of SEQ ID NO:25, b is an integer of 15 to 900, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:25, and where b is greater than or equal to a + 14.	AA058407
550118	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1308 of SEQ ID NO:26, b is an integer of 15 to 1322, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:26, and where b is greater than or equal to a + 14.	
550148	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 443 of SEQ ID NO:27, b is an integer of 15 to 457, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:27, and where b is greater than or equal to a + 14.	
550870	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 582 of SEQ ID NO:28, b is an integer of 15 to 596, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:28, and where b is greater than or equal to a + 14.	

552506	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 422 of SEQ ID NO:29, b is an integer of 15 to 436, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:29, and where b is greater than or equal to a + 14.	
553765	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1300 of SEQ ID NO:30, b is an integer of 15 to 1314, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:30, and where b is greater than or equal to a + 14.	T56196, T60502, T68045, T68126, T68223, T68924, T68956, T69698, T70509, T71155, T72771, T73042, T74806, H47199, H93928
554050	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1453 of SEQ ID NO:31, b is an integer of 15 to 1467, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:31, and where b is greater than or equal to a + 14.	T47267, T71354, R60150, R73621, H61209, H61252, H61301, H62114, W73095, AA100106
554186	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2332 of SEQ ID NO:32, b is an integer of 15 to 2346, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:32, and where b is greater than or equal to a + 14.	
554716	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 445 of SEQ ID NO:33, b is an integer of 15 to 459, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:33, and where b is greater than or equal to a + 14.	AA155695
556791	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 615 of SEQ ID NO:34, b is an integer of 15 to 629, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:34, and where b is greater than or equal to a + 14.	
557121	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 904 of SEQ ID NO:35, b is an integer of 15 to 918, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:35, and where b is greater than or equal to a + 14.	R64392, N79271, N93935, W40435, W94836, AA032255, AA033626, AA043229, AA043230, AA150687, AA150859
557199	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 788 of SEQ ID NO:36, b is an integer of 15 to 802, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:36, and where b is greater than or equal to a + 14.	
557293	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2079 of SEQ ID NO:37, b is an integer of 15 to 2093, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:37, and where b is greater than or equal to a + 14.	N52364, N75135, N75421, W05142, W07655, AA029997, AA029107, AA463728
557441	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 420 of SEQ ID NO:38, b is an integer of 15 to 434, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:38, and where b is greater than or equal to a + 14.	
558091	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1064 of SEQ ID NO:39, b is an integer of 15 to 1078, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:39, and where b is greater than or equal to a + 14.	
558423	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1962 of SEQ ID NO:40, b is an integer of 15 to 1976, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:40, and where b is greater than or equal to a + 14.	T49968, R56305, H08010, H47549, N29144, N39902, N77470, N78717, AA182657, AA242919, AA252178
558465	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2296 of SEQ ID NO:41, b is an integer of 15 to 2310, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:41, and where b is greater than or equal to a + 14.	T85937, T96679, T96794, R13767, R14771, R38610, R42541, R42541, R60238, R60472, H14363, H14409, R94149, N30062, N30065, N40770, N92651, N92649, N99584, N99587, W37817
558493	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 392 of SEQ ID NO:42, b is an integer of 15 to 406, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:42, and where b is greater than or equal to a + 14.	
558778	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	

	sequence described by the general formula of a-b, where a is any integer between 1 to 613 of SEQ ID NO:43, b is an integer of 15 to 627, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:43, and where b is greater than or equal to a + 14.	
558818	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 731 of SEQ ID NO:44, b is an integer of 15 to 745, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:44, and where b is greater than or equal to a + 14.	
563182	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 453 of SEQ ID NO:45, b is an integer of 15 to 467, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:45, and where b is greater than or equal to a + 14.	
572571	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 708 of SEQ ID NO:46, b is an integer of 15 to 722, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:46, and where b is greater than or equal to a + 14.	R07415, R02207, H14209
575525	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 988 of SEQ ID NO:47, b is an integer of 15 to 1002, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:47, and where b is greater than or equal to a + 14.	R52330, H20661
580659	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2105 of SEQ ID NO:48, b is an integer of 15 to 2119, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:48, and where b is greater than or equal to a + 14.	
583650	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 480 of SEQ ID NO:49, b is an integer of 15 to 494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:49, and where b is greater than or equal to a + 14.	
584698	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b,	

	where a is any integer between 1 to 1328 of SEQ ID NO:50, b is an integer of 15 to 1342, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:50, and where b is greater than or equal to a + 14.	
585791	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1513 of SEQ ID NO:51, b is an integer of 15 to 1527, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:51, and where b is greater than or equal to a + 14.	T48321, T67802, T67948, T67040, T67041, T83908, R09529, R09642, T83737, R16473, R16773, R25443, R26269, H05343, H26912, H28048, H39855, R86113, N33097, N44668, N79489, W16656, W60696, W60757, AA081126, AA081151, AA083763, AA132950, AA132862, AA149302, AA149416, AA191527, AA194936, AA195535, AA233905, AA234134
587229	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 616 of SEQ ID NO:52, b is an integer of 15 to 630, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:52, and where b is greater than or equal to a + 14.	
587246	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 561 of SEQ ID NO:53, b is an integer of 15 to 575, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:53, and where b is greater than or equal to a + 14.	
587486	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2920 of SEQ ID NO:54, b is an integer of 15 to 2934, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:54, and where b is greater than or equal to a + 14.	T71052, T71121, T72185, R21828, R21895, N51506, N53649, N66770, W72635, W77877, AA063260, AA083833, AA165549, AA165652, AA169616, AA256205, AA256348, AA464908
589218	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 561 of SEQ ID NO:55, b is an integer of 15 to 575, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:55, and where b is greater than or equal to a + 14.	R31110, N36905, N36910, N48189, W32216, AA069678, AA173954
592154	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1126 of SEQ ID NO:56, b is an integer of 15 to 1140, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:56, and where b is greater than or equal to a + 14.	R12094, T66653, T80236, R15999, R25029, R35910, AA194354
598664	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	W40222

	sequence described by the general formula of a-b, where a is any integer between 1 to 241 of SEQ ID NO:57, b is an integer of 15 to 255, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:57, and where b is greater than or equal to a + 14.	
598665	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1240 of SEQ ID NO:58, b is an integer of 15 to 1254, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:58, and where b is greater than or equal to a + 14.	W39277, W39349, W39357, W39764, W39767, W40288, W40538, W44820, W45264, W51936, W51937, W51918, W52848, W74327
604719	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1176 of SEQ ID NO:59, b is an integer of 15 to 1190, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:59, and where b is greater than or equal to a + 14.	T49228, T49490, T70505, T70428, T73981, T86568, T86746, T91867, R10309, R12088, T79988, T80222, T84402, T85263, T85576, T85577, R05432, R13226, R13278, R13833, R18842, R19462, R21598, R22718, R35298, H10723, H11136, H44767, R88961, R92868, R92897, R97874, H71254, H71922, H78937, H79825, H79920, H80125, H86893, H90187, N25116, N44644, N50007, N53591, N72554, W40421, W42525, W52370, AA021224, AA037505, AA053988
612689	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 566 of SEQ ID NO:60, b is an integer of 15 to 580, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:60, and where b is greater than or equal to a + 14.	H54589, AA227410
612980	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 439 of SEQ ID NO:61, b is an integer of 15 to 453, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:61, and where b is greater than or equal to a + 14.	
615134	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2579 of SEQ ID NO:62, b is an integer of 15 to 2593, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:62, and where b is greater than or equal to a + 14.	T54861, T55025, T92712, T92716, T92721, T92789, T92795, T92801, T92938, T93055, T93331, T94009, R15352, R25472, R26297, R33615, R33726, R53088, R62766, R62767, R71478, R71526, R78919, R79016, H06272, H06317, H24935, H24973, H28559, H28560, H42644, H38452, H38491, H47593, H47673, R87481, R88156, R89767, R89789, H51597, H57134, H57205, H62215, H62312, H97605, N24503, N27658, N35013, N43767, N92918, W15223, W39515, W72421, W76280, W86384,

		AA031688, AA031689, AA036840, AA045285, AA046566, AA099284, AA132058, AA132202, AA150688, AA150860, AA156675, AA159469, AA160880, AA165451, AA165638, AA173528, AA173712, AA458903, AA459097
616064	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1181 of SEQ ID NO:63, b is an integer of 15 to 1195, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:63, and where b is greater than or equal to a + 14.	
616096	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 378 of SEQ ID NO:64, b is an integer of 15 to 392, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:64, and where b is greater than or equal to a + 14.	
616926	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1276 of SEQ ID NO:65, b is an integer of 15 to 1290, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:65, and where b is greater than or equal to a + 14.	AA149936, AA150476, AA167701, AA167815, AA256842, AA256431, AA458750
634923	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 702 of SEQ ID NO:66, b is an integer of 15 to 716, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:66, and where b is greater than or equal to a + 14.	
646688	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1112 of SEQ ID NO:67, b is an integer of 15 to 1126, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:67, and where b is greater than or equal to a + 14.	
647531	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2125 of SEQ ID NO:68, b is an integer of 15 to 2139, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:68, and where b is greater than or equal to a + 14.	
647695	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	W52753, W60008, W60952, W73125

	sequence described by the general formula of a-b, where a is any integer between 1 to 1327 of SEQ ID NO:69, b is an integer of 15 to 1341, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:69, and where b is greater than or equal to a + 14.	
647699	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 721 of SEQ ID NO:70, b is an integer of 15 to 735, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:70, and where b is greater than or equal to a + 14.	
651706	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2016 of SEQ ID NO:71, b is an integer of 15 to 2030, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:71, and where b is greater than or equal to a + 14.	T71695, T71768, R08204, R08255, R31484, R31485, R50842, R52642, R53297, R60059, R60122, R60247, R60760, R62567, R62568, R70726, R71415, H38156, R83081, R94374, R94394, H53235, H60439, H60485, H63520, H63921, H64892, H65484, H71929, H77840, H77887, H78275, H79162, H80573, H94710, H95076, H95259, H95309, N46854, N47172, N49873, N55275, N64845, N68747, N74193, N74236, N91640, W01175, W01240, W57593, AA129298, AA129339, AA133183, AA133370
651726	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1861 of SEQ ID NO:72, b is an integer of 15 to 1875, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:72, and where b is greater than or equal to a + 14.	T90733, R10849, R10850, T82138, T83264, R87054, R91713, H71337, H71389, H72382, N55250, N74908, N76660, N76857, W20174, W23436, W35129, AA045320, AA045221
652160	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 846 of SEQ ID NO:73, b is an integer of 15 to 860, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:73, and where b is greater than or equal to a + 14.	
654015	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 506 of SEQ ID NO:74, b is an integer of 15 to 520, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:74, and where b is greater than or equal to a + 14.	
656339	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 849 of SEQ ID NO:75, b is an integer of 15 to 863, where both a and	H70078

	b correspond to the positions of nucleotide residues shown in SEQ ID NO:75. and where b is greater than or equal to a + 14.	
657190	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 677 of SEQ ID NO:76. b is an integer of 15 to 691. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:76. and where b is greater than or equal to a + 14.	
657859	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 311 of SEQ ID NO:77. b is an integer of 15 to 325. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:77. and where b is greater than or equal to a + 14.	
662143	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 807 of SEQ ID NO:78. b is an integer of 15 to 821. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:78. and where b is greater than or equal to a + 14.	R27497. R33219. R94577. N95517
662212	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 603 of SEQ ID NO:79. b is an integer of 15 to 617. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:79, and where b is greater than or equal to a + 14.	
662225	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1175 of SEQ ID NO:80. b is an integer of 15 to 1189. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:80. and where b is greater than or equal to a + 14.	R59488. H11016. N29502. AA025624
662496	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 452 of SEQ ID NO:81. b is an integer of 15 to 466. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:81, and where b is greater than or equal to a + 14.	
669529	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 346 of SEQ ID NO:82. b is an integer of 15 to 360. where both a and b correspond to the positions of nucleotide residues	

	shown in SEQ ID NO:82. and where b is greater than or equal to a + 14.	
670453	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2095 of SEQ ID NO:83. b is an integer of 15 to 2109. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:83. and where b is greater than or equal to a + 14.	T77608. R09248. R09364. R11470. R19371. R39244. H15039. H15949. H27001. H30603. H37983. R84579. R85044. R85045. R85469. H85744. H99185. N24468. N52798. N68992. N76620. W15294. W39329. W52841. W95437. W95781. AA057725. AA059439
675028	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1521 of SEQ ID NO:84. b is an integer of 15 to 1535. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:84. and where b is greater than or equal to a + 14.	T48289, T77554, R05507. R25405. R31496. R32660. R41978. R41978. R62600. R62648. R63390. R63445. R68659. R68711, R68771, R68865. H01655. H01656. H04239. R92875. R93091. H83742. H83886. H89969. N30705. N64395. N64408. N66492. N67310. N68265. N80959. N92190. W79008. W80400. N90831. AA075349. AA075461. AA224356
681325	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 417 of SEQ ID NO:85. b is an integer of 15 to 431. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:85. and where b is greater than or equal to a + 14.	
683103	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1128 of SEQ ID NO:86. b is an integer of 15 to 1142. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:86. and where b is greater than or equal to a + 14.	
684432	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1783 of SEQ ID NO:87. b is an integer of 15 to 1797. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:87. and where b is greater than or equal to a + 14.	R52639. R53294. R71671. R71703. H40352. H40408. R96789. R97034. R97271. R97719. H49468. H49467. H56659. H56739. H59341. H59998. H63308. H93861. H94642. H94643. N30295. N31741. N31742. N42019. N42450. N53570. N53844. N63677. N64865. N70725. N72529. N73341. N92110. N92116. N99031. W16862. W39154. W86457. N89634. AA005356. AA007379. AA235011. AA236270. AA253267
688018	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 367 of SEQ ID NO:88. b is an integer of 15 to 381. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:88. and where b is greater than or equal to a + 14.	T54297
688077	Preferably excluded from the present invention are	H00845. H01228. R95095. N76784.

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 524 of SEQ ID NO:89, b is an integer of 15 to 538, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:89, and where b is greater than or equal to a + 14.	N98607, W24232, W52082, W56721, W56767, W67714, W68173, W90739, W90774, AA033634, AA034341, AA062620, AA062994, AA258212
691522	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2107 of SEQ ID NO:90, b is an integer of 15 to 2121, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:90, and where b is greater than or equal to a + 14.	
693706	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2960 of SEQ ID NO:91, b is an integer of 15 to 2974, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:91, and where b is greater than or equal to a + 14.	T78218, T81634, R13915, R18080, H18055, H63131, H67579, AA035361, AA069801, AA069848, AA076182, AA079495, AA082095, AA102007, AA100775, AA143208, AA143346, AA146711, AA147300, AA180012, AA235098
694523	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 398 of SEQ ID NO:92, b is an integer of 15 to 412, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:92, and where b is greater than or equal to a + 14.	
697517	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1869 of SEQ ID NO:93, b is an integer of 15 to 1883, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:93, and where b is greater than or equal to a + 14.	T90609, AA053480, AA074689, AA102775, AA122090, AA182511, AA243116
699054	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2297 of SEQ ID NO:94, b is an integer of 15 to 2311, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:94, and where b is greater than or equal to a + 14.	
699464	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 500 of SEQ ID NO:95, b is an integer of 15 to 514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:95, and where b is greater than or equal to a + 14.	T82960
703402	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	

	sequence described by the general formula of a-b. where a is any integer between 1 to 451 of SEQ ID NO:96. b is an integer of 15 to 465. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:96. and where b is greater than or equal to a + 14.	
703651	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1445 of SEQ ID NO:97. b is an integer of 15 to 1459. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:97. and where b is greater than or equal to a + 14.	
704905	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 865 of SEQ ID NO:98. b is an integer of 15 to 879. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:98. and where b is greater than or equal to a + 14.	
706907	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 234 of SEQ ID NO:99. b is an integer of 15 to 248. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:99. and where b is greater than or equal to a + 14.	
708515	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 466 of SEQ ID NO:100. b is an integer of 15 to 480. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:100. and where b is greater than or equal to a + 14.	R35145. H20357. H25361. H40070. N24435. N56688. W92201. AA164775
710572	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 439 of SEQ ID NO:101. b is an integer of 15 to 453. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:101, and where b is greater than or equal to a + 14.	AA188988. AA188989
710618	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 889 of SEQ ID NO:102. b is an integer of 15 to 903, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:102. and where b is greater than or equal to a + 14.	T92687, N50744
711810	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b.	

	where a is any integer between 1 to 1774 of SEQ ID NO:103, b is an integer of 15 to 1788, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:103, and where b is greater than or equal to a + 14.	
714933	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3305 of SEQ ID NO:104, b is an integer of 15 to 3319, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:104, and where b is greater than or equal to a + 14.	T65559, T65626, R10350, R13281, R13569, R14670, R15257, R34544, R36053, R39872, R40726, R49062, R49135, R53355, R53957, R49062, R49135, R40726, R78042, H05306, H05356, H07035, H10902, H14308, H24047, H24154, R89696, R93433, R98651, R98650, H50887, H53389, H91935, H91944, H99472, N25040, N26192, N28285, N48283, N49011, N62360, N68609, N71824, N79127, W72510, W76067, W94862, W94822, W96008, W96040, AA025005, AA036767, AA044132, AA044098, AA047829, AA047855, AA054452, AA054567, AA057171, AA085624, AA088811, AA130768, AA130944, AA132373, AA132618, AA150892, AA151019, AA157288, AA157368, AA157369, AA159896, AA160826, AA180535, AA187424, AA187614
716331	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1972 of SEQ ID NO:105, b is an integer of 15 to 1986, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:105, and where b is greater than or equal to a + 14.	
717686	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 577 of SEQ ID NO:106, b is an integer of 15 to 591, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:106, and where b is greater than or equal to a + 14.	
718187	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 139 of SEQ ID NO:107, b is an integer of 15 to 153, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:107, and where b is greater than or equal to a + 14.	
719934	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1522 of SEQ ID NO:108, b is an integer of 15 to 1536, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:108, and where b is	

	greater than or equal to a + 14.	
722980	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 498 of SEQ ID NO:109, b is an integer of 15 to 512, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:109, and where b is greater than or equal to a + 14.	
723596	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1441 of SEQ ID NO:110, b is an integer of 15 to 1455, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:110, and where b is greater than or equal to a + 14.	W90706, W95592, AA047652, AA250970, AA250874, AA251071, AA251074, AA251073
724352	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 661 of SEQ ID NO:111, b is an integer of 15 to 675, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:111, and where b is greater than or equal to a + 14.	
724450	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 534 of SEQ ID NO:112, b is an integer of 15 to 548, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:112, and where b is greater than or equal to a + 14.	
724855	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 462 of SEQ ID NO:113, b is an integer of 15 to 476, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:113, and where b is greater than or equal to a + 14.	T77137, T88762, T99291, R07006, AA004532
724904	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1002 of SEQ ID NO:114, b is an integer of 15 to 1016, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:114, and where b is greater than or equal to a + 14.	
725642	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 480 of SEQ ID NO:115, b is an integer of 15 to 494, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:115, and where b is greater than or equal to a + 14.	

726192	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 3222 of SEQ ID NO:116. b is an integer of 15 to 3236. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:116. and where b is greater than or equal to a + 14.	T65882. T66040. T77662. R53239, R59914. R59915, R62156, R62264, R63487. H04945. H04951. H13535, H13536. H16274. N25318. N25787. N31430, N32153. N36498. N49086, N49333. N50212. N66885. N78949. AA115267. AA115291. AA150461. AA164418. AA195130. AA195277. AA234969. AA236191. AA251324. AA251530. AA251517, AA258562, AA258724
726964	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 897 of SEQ ID NO:117. b is an integer of 15 to 911, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:117. and where b is greater than or equal to a + 14.	
730930	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1963 of SEQ ID NO:118, b is an integer of 15 to 1977. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:118. and where b is greater than or equal to a + 14.	
731314	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 790 of SEQ ID NO:119. b is an integer of 15 to 804. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:119. and where b is greater than or equal to a + 14.	R32598. R36499
732386	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 723 of SEQ ID NO:120. b is an integer of 15 to 737. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:120. and where b is greater than or equal to a + 14.	AA417877. AA424537. AA424604
732909	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1238 of SEQ ID NO:121. b is an integer of 15 to 1252, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:121. and where b is greater than or equal to a + 14.	
733088	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1834 of SEQ ID NO:122. b is an integer of 15 to 1848. where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:122, and where b is greater than or equal to a + 14.	
733351	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 449 of SEQ ID NO:123, b is an integer of 15 to 463, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:123, and where b is greater than or equal to a + 14.	
733693	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 336 of SEQ ID NO:124, b is an integer of 15 to 350, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:124, and where b is greater than or equal to a + 14.	
734760	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1570 of SEQ ID NO:125, b is an integer of 15 to 1584, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:125, and where b is greater than or equal to a + 14.	T78350, T79874, R13714, H83297, H86534, N20546, N93623, N93932, W23965, AA016035, AA016080, AA017047, AA021630, AA046286, AA063218, AA076542, AA158847, AA159397, AA160406, AA213767, AA255605, AA422075, AA421997, AA424997
735711	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1290 of SEQ ID NO:126, b is an integer of 15 to 1304, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:126, and where b is greater than or equal to a + 14.	
742413	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 887 of SEQ ID NO:127, b is an integer of 15 to 901, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:127, and where b is greater than or equal to a + 14.	R99084, R99627
742676	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3273 of SEQ ID NO:128, b is an integer of 15 to 3287, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:128, and where b is greater than or equal to a + 14.	T40095, T40106, T40156, T41006, T46845, T46862, T46892, T51126, T51142, T51176, T51197, T53736, T53747, T53827, T53835, T53850, T53939, T53959, T55991, T56035, T56068, T56236, T56378, T57000, T57001, T58101, T58719, T58786, T58850, T58866, T58898, T58906, T58910, T58925, T58961, T60324, T60332, T60352, T60362, T60377, T60385, T60424, T60444, T60476, T60477, T60507, T60570, T60599, T60631, T61109, T61277, T61376, T61409, T61618, T61702, T61743, T61865, T61875, T62046, T62079,

		T62110, T62136, T39959, T47778, T47810, T53910, T61195, T61199, T61883, T62738, T62764, T62888, T62914, T64121, T64186, T64232, T64242, T64305, T64309, T64585, T64595, T64652, T64692, T64696, T64738, T64751, T67432, T67593, T67633, T67703, T67725, T67736, T67739, T67753, T67755, T67820, T67837, T67845, T67848, T67862, T67864, T67886, T67895, T67907, T67922, T67929, T67971, T68044, T68055, T68070, T68106, T68107, T68170, T68176, T68201, T68220, T68245, T68267, T68291, T68301, T68329, T68355, T68367, T68401, T68516, T68607, T68688, T68716, T68772, T68781, T68842, T68914, T69001, T69031, T69081, T69122, T69139, T69145, T69180, T69197, T69206, T69230, T69243, T69283, T69293, T69317, T69358, T69368, T69400, T69420, T69445, T70452, T70475, T70494, T70495, T70498, T70975, T71039, T71105, T71313, T71351, T71356, T71429, T71457, T71518, T71692, T71698, T71712, T71715, T71781, T71784, T71800, T71851, T71857, T71870, T71875, T71895, T71908, T71914, T71916, T71959, T72031, T72037, T72042, T72063, T72065, T72079, T72098, T72099, T72152, T72177, T72178, T72199, T72223, T72300, T72304, T72360, T72394, T72407, T72418, T72451, T72456, T72464, T72510, T72517, T72525, T72793, T72803, T72821, T72826, T72827, T72956, T72957, T72978, T73010, T73052, T73096, T73203, T73225, T73250, T73258, T73265, T73317, T73333, T73382, T73400, T73410, T73425, T73427, T73445, T73493, T73495, T73512, T73566, T73666, T73729, T73768, T73787, T73819, T73868, T73873, T73920, T73931, T73952, T73962, T74033, T74101, T74111, T74269, T74273, T74372, T74380, T74407, T74474, T74485, T74541, T74598, T74615, T74645, T74658, T74673, T74677, T74756, T74765, T74843, T74854, T74860, T74863, T74914, T71341, T71501, T77799, T90078, T82897, T95610, T95711, R02292, R02293, R06796, R95746, R98475, H48262, H48353, H58120, H58121, H61463, H67459, H70620,

		H90426, H90482, H94389, N33594, N49440, N75535, W05328, W19064, W86031, AA011414, AA026625, AA026737, AA235252
742781	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1668 of SEQ ID NO:129, b is an integer of 15 to 1682, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:129, and where b is greater than or equal to a + 14.	R00982, R00983, R20611, R21647, R46119, R46119, H29203, H29204, N47470, N47471, N64818, N75670, N79512, N92805, W16709, AA023019, AA022493, AA143187, AA171546, AA233410, AA460731
743356	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 286 of SEQ ID NO:130, b is an integer of 15 to 300, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:130, and where b is greater than or equal to a + 14.	
745694	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 91 of SEQ ID NO:131, b is an integer of 15 to 105, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:131, and where b is greater than or equal to a + 14.	
747235	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 897 of SEQ ID NO:132, b is an integer of 15 to 911, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:132, and where b is greater than or equal to a + 14.	
750986	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3562 of SEQ ID NO:133, b is an integer of 15 to 3576, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:133, and where b is greater than or equal to a + 14.	
751068	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1179 of SEQ ID NO:134, b is an integer of 15 to 1193, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:134, and where b is greater than or equal to a + 14.	W23633, W35271, W86390, W86391
751164	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1931 of SEQ ID NO:135, b is an integer of 15 to 1945, where both a	

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:135, and where b is greater than or equal to a + 14.	
751890	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1132 of SEQ ID NO:136, b is an integer of 15 to 1146, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:136, and where b is greater than or equal to a + 14.	R12199, AA056402
751991	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2331 of SEQ ID NO:137, b is an integer of 15 to 2345, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:137, and where b is greater than or equal to a + 14.	
752449	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 717 of SEQ ID NO:138, b is an integer of 15 to 731, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:138, and where b is greater than or equal to a + 14.	H49093, H63940, H68327, H72930, H80397, N59075, N59482
752504	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 743 of SEQ ID NO:139, b is an integer of 15 to 757, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:139, and where b is greater than or equal to a + 14.	
752688	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 649 of SEQ ID NO:140, b is an integer of 15 to 663, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:140, and where b is greater than or equal to a + 14.	T83204, W07391
752889	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3921 of SEQ ID NO:141, b is an integer of 15 to 3935, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:141, and where b is greater than or equal to a + 14.	
753150	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2198 of SEQ ID NO:142, b is an integer of 15 to 2212, where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:142. and where b is greater than or equal to a + 14.	
753690	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 729 of SEQ ID NO:143. b is an integer of 15 to 743. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:143. and where b is greater than or equal to a + 14.	AA262521
754479	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 825 of SEQ ID NO:144. b is an integer of 15 to 839. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:144. and where b is greater than or equal to a + 14.	
754692	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2893 of SEQ ID NO:145, b is an integer of 15 to 2907. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:145. and where b is greater than or equal to a + 14.	
756814	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1823 of SEQ ID NO:146, b is an integer of 15 to 1837. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:146. and where b is greater than or equal to a + 14.	T51378. T54439. T54440, T54492, T39385. T89470, T89560. R05534, R05644. R17667. R25313. R32922, R33132. R33284, R35666, R35777, R38043, R38132, R38752. R43414, R54027. R54028. R43414, R63780, R64328. R64614. R64615. R74563. R82622, H01362. H01835. H02683, H02973. H04269. H09641. H09675. H10002. H13064. H13271. H13720. H13933. H13934, H15328. H15712. H15993, R83464. R83844. R83845, R89553, R95676, R97388. R98691. R98917, H48613. H48805. H51096, H51682. H58872. H58873. H67326, H68534, H70197. H78192. H78193, H79697. H79698. H83266. H83267, H90205. H90308, H90862. H90962, H94344, H95788. H96137. H97956, H99868, N28553. N68855. N94629, W31434, W31994. W46421. W52814, W56529. W56780. W58375. W58549, W58662, W68203. W68204. W69142, W69248, W81130. W81131. W81700, W81701. AA043367. AA043368. AA044067. AA044159. AA122334, AA464398. AA419080. AA423821. AA428882. AA428973. AA429196
757127	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b.	

	where a is any integer between 1 to 1357 of SEQ ID NO:147, b is an integer of 15 to 1371, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:147, and where b is greater than or equal to a + 14.	
757347	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1743 of SEQ ID NO:148, b is an integer of 15 to 1757, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:148, and where b is greater than or equal to a + 14.	
757495	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3518 of SEQ ID NO:149, b is an integer of 15 to 3532, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:149, and where b is greater than or equal to a + 14.	R24543, R24651, R25091, R36580, R45429, R51961, R53628, R45429, R64120, R64218, R67926, R69338, R69339, R74200, R74291, R80166, H00661, H00753, H02579, H02665, H64801, H64802, H64802, N63215, N75662, W46814, W46864, W70290, W72831, W72832, W75986, W90099, W90197, AA025841, AA025842, AA039870, AA040233, AA043893, AA042891, AA043018, AA062769, AA074082, AA075813, AA082428, AA196448, AA196691
757715	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1917 of SEQ ID NO:150, b is an integer of 15 to 1931, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:150, and where b is greater than or equal to a + 14.	R10018, T80752, T81225, R13945, H14918, H45144, N78192, W01185, W52734, W73106, W79308, AA043840, AA044358, AA064738, AA160313, AA196613, AA226860, AA232389
760388	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1617 of SEQ ID NO:151, b is an integer of 15 to 1631, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:151, and where b is greater than or equal to a + 14.	T71835, T94624, T82230, T96710, R23486, R23859, R26080, R36711, R37553, R38131, H87609, N26790, N41457, W24534, W31754, W31873, W32038, W32317, W32647, W38857, W39517, W39338, W56012, W56108, W56683, W57744, W72389, W76407, W93884, W93885, AA010989, AA160043, AA169520
760433	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 718 of SEQ ID NO:152, b is an integer of 15 to 732, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:152, and where b is greater than or equal to a + 14.	
760545	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 480 of SEQ ID NO:153, b is an integer of 15 to 494, where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:153, and where b is greater than or equal to a + 14.	
761566	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2427 of SEQ ID NO:154, b is an integer of 15 to 2441, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:154, and where b is greater than or equal to a + 14.	T69288, T69363, T94926, R12359, R26909, R27151, R37284, R61007, R61674, R68776, R68872, R70952, R71004, H92792, H92913, N25506, N32325, N57420, N68341, N94012, AA011440, AA076005, AA076006, AA129646, AA129781, AA187676
761740	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2933 of SEQ ID NO:155, b is an integer of 15 to 2947, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:155, and where b is greater than or equal to a + 14.	R13217, R30963, R31018, R40301, R51543, R51544, R40301, R63409, H29530, H83725, H98067, N20307, N27578, N28375, N46832, N62348, N62593, N78359, N79110, AA041460, AA041513, AA046252, AA046371, AA125849, AA125850, AA252450, AA461403
765215	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 652 of SEQ ID NO:156, b is an integer of 15 to 666, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:156, and where b is greater than or equal to a + 14.	T54662, T54749
765428	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 613 of SEQ ID NO:157, b is an integer of 15 to 627, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:157, and where b is greater than or equal to a + 14.	
766686	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 888 of SEQ ID NO:158, b is an integer of 15 to 902, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:158, and where b is greater than or equal to a + 14.	
767396	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 579 of SEQ ID NO:159, b is an integer of 15 to 593, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:159, and where b is greater than or equal to a + 14.	AA172282, AA220915
767501	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1833 of SEQ ID NO:160, b is an integer of 15 to 1847, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:160, and where b is	T48254, T48253, T61610, T61695, T70390, T70397, T86348, R11405, R05486, R05593, R19155, R61228, R61229, R70142, R70143, R78897, R78993, R94037, N81160, W90480, W90479, W95079, AA192429

	greater than or equal to a + 14.	
767945	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 356 of SEQ ID NO:161, b is an integer of 15 to 370, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:161, and where b is greater than or equal to a + 14.	
768996	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 440 of SEQ ID NO:162, b is an integer of 15 to 454, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:162, and where b is greater than or equal to a + 14.	
771415	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1082 of SEQ ID NO:163, b is an integer of 15 to 1096, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:163, and where b is greater than or equal to a + 14.	
772657	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2009 of SEQ ID NO:164, b is an integer of 15 to 2023, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:164, and where b is greater than or equal to a + 14.	T39789, R21583, R23570, R63603, R63604, R80168, R80167, H02287, H02391, N25705, N26310, N26346, N34095, N39754, N51681, N91936, W24114, AA035390, AA035389, AA043307, AA043308, AA043279, AA043280, AA053303, AA058551, AA082488, AA122113, AA142961, AA149350, AA149351, AA150613, AA150739, AA150847, AA179036, AA251541, AA251499
773123	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1306 of SEQ ID NO:165, b is an integer of 15 to 1320, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:165, and where b is greater than or equal to a + 14.	
773193	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1191 of SEQ ID NO:166, b is an integer of 15 to 1205, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:166, and where b is greater than or equal to a + 14.	
773710	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1399 of SEQ ID NO:167, b is an integer of 15 to 1413, where both a	T91089, T84760, R18409, R42472, R44656, R42472, R44656, R70650, H94730, H94759, N30658, N66021, N66027, N66688, N95136, N98956, AA131377, AA131494, AA131593,

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:167, and where b is greater than or equal to a + 14.	AA131658, AA227712, AA227958, AA424025
774283	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1214 of SEQ ID NO:168, b is an integer of 15 to 1228, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:168, and where b is greater than or equal to a + 14.	R81621, H75455, H75454, AA165108, AA164711, AA461410, AA461095
774369	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1911 of SEQ ID NO:169, b is an integer of 15 to 1925, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:169, and where b is greater than or equal to a + 14.	R26376, R66765, H86185, AA016184, AA021102, AA028914, AA133277, AA133354
774754	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1544 of SEQ ID NO:170, b is an integer of 15 to 1558, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:170, and where b is greater than or equal to a + 14.	W38589, W74674, W74780, N90213, AA043957, AA043823, AA157016
774823	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1388 of SEQ ID NO:171, b is an integer of 15 to 1402, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:171, and where b is greater than or equal to a + 14.	
775510	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 476 of SEQ ID NO:172, b is an integer of 15 to 490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:172, and where b is greater than or equal to a + 14.	
775634	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1423 of SEQ ID NO:173, b is an integer of 15 to 1437, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:173, and where b is greater than or equal to a + 14.	
775640	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1801 of SEQ ID NO:174, b is an integer of 15 to 1815, where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:174, and where b is greater than or equal to a + 14.	
775802	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 957 of SEQ ID NO:175, b is an integer of 15 to 971, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:175, and where b is greater than or equal to a + 14.	
777470	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1608 of SEQ ID NO:176, b is an integer of 15 to 1622, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:176, and where b is greater than or equal to a + 14.	R72009, R81577, H26684, H45155, R87903, R87922, W46492, W51858
777652	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 326 of SEQ ID NO:177, b is an integer of 15 to 340, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:177, and where b is greater than or equal to a + 14.	
778998	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 602 of SEQ ID NO:178, b is an integer of 15 to 616, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:178, and where b is greater than or equal to a + 14.	
779273	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2053 of SEQ ID NO:179, b is an integer of 15 to 2067, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:179, and where b is greater than or equal to a + 14.	
779297	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1813 of SEQ ID NO:180, b is an integer of 15 to 1827, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:180, and where b is greater than or equal to a + 14.	T58639, T58688, T65114, T65181, T79935, R37097, H01720, H93130, N49316, N49558, W32803, W95634, AA025739, AA426310, AA428778
779664	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2012 of SEQ ID NO:181, b is an integer of 15 to 2026, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:181, and where b is	T91627, R18325, R37374, R59694, R60216, R60450, H28798, H28818, N30799, N39412, W74507, W79219, AA083583, AA135148, AA164254, AA164365, AA172128

	greater than or equal to a + 14.	
780565	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 442 of SEQ ID NO:182, b is an integer of 15 to 456, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:182, and where b is greater than or equal to a + 14.	
780665	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 467 of SEQ ID NO:183, b is an integer of 15 to 481, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:183, and where b is greater than or equal to a + 14.	W60277
780666	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 482 of SEQ ID NO:184, b is an integer of 15 to 496, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:184, and where b is greater than or equal to a + 14.	
781579	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1293 of SEQ ID NO:185, b is an integer of 15 to 1307, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:185, and where b is greater than or equal to a + 14.	T57785, T82345, W86564, AA078858, AA155901, AA161451, AA178927, AA194606
782052	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 435 of SEQ ID NO:186, b is an integer of 15 to 449, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:186, and where b is greater than or equal to a + 14.	
782393	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 937 of SEQ ID NO:187, b is an integer of 15 to 951, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:187, and where b is greater than or equal to a + 14.	N25688, N30017, N34076, N36364, N46861, N47181, N62606, N92811, W24930, W25337, W47158, W47279, W49821, AA234682, AA234755, AA252206
782907	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 367 of SEQ ID NO:188, b is an integer of 15 to 381, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:188, and where b is greater than or equal to a + 14.	

783220	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1295 of SEQ ID NO:189, b is an integer of 15 to 1309, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:189, and where b is greater than or equal to a + 14.	
783300	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1885 of SEQ ID NO:190, b is an integer of 15 to 1899, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:190, and where b is greater than or equal to a + 14.	
783938	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2476 of SEQ ID NO:191, b is an integer of 15 to 2490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:191, and where b is greater than or equal to a + 14.	
784024	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1794 of SEQ ID NO:192, b is an integer of 15 to 1808, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:192, and where b is greater than or equal to a + 14.	H89685, N20336, N27611, N31596, N42655, N51849, N51859, N62943, AA236316, AA253217
784575	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1059 of SEQ ID NO:193, b is an integer of 15 to 1073, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:193, and where b is greater than or equal to a + 14.	
785006	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 373 of SEQ ID NO:194, b is an integer of 15 to 387, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:194, and where b is greater than or equal to a + 14.	
785069	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 959 of SEQ ID NO:195, b is an integer of 15 to 973, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:195, and where b is greater than or equal to a + 14.	
785237	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 629 of SEQ ID NO:196, b is an integer of 15 to 643, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:196, and where b is greater than or equal to a + 14.	
786111	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 438 of SEQ ID NO:197, b is an integer of 15 to 452, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:197, and where b is greater than or equal to a + 14.	
787036	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1018 of SEQ ID NO:198, b is an integer of 15 to 1032, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:198, and where b is greater than or equal to a + 14.	R11814, H14163, N42713, W69844, AA076578
788991	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2718 of SEQ ID NO:199, b is an integer of 15 to 2732, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:199, and where b is greater than or equal to a + 14.	T94446, T94533, R12065, R13249, R13635, R38488, R40329, R43592, R46434, R43592, R40329, H16332, H20990, H28489, H29906, H39987, R83899, R85669, R85905, H57115, H89691, W01303, W03530, W44921, W52157, AA001492, AA001493, AA054074, AA054263, AA059205, AA059263, AA461201, AA461378, AA417279, AA417269, AA429343
789125	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2301 of SEQ ID NO:200, b is an integer of 15 to 2315, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:200, and where b is greater than or equal to a + 14.	
789626	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 876 of SEQ ID NO:201, b is an integer of 15 to 890, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:201, and where b is greater than or equal to a + 14.	
789703	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1519 of SEQ ID NO:202, b is an integer of 15 to 1533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:202, and where b is greater than or equal to a + 14.	

789858	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2812 of SEQ ID NO:203, b is an integer of 15 to 2826, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:203, and where b is greater than or equal to a + 14.	
790848	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1524 of SEQ ID NO:204, b is an integer of 15 to 1538, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:204, and where b is greater than or equal to a + 14.	R62582, R62583, N45584, N48793, N49502
790893	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2328 of SEQ ID NO:205, b is an integer of 15 to 2342, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:205, and where b is greater than or equal to a + 14.	
790912	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 813 of SEQ ID NO:206, b is an integer of 15 to 827, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:206, and where b is greater than or equal to a + 14.	T79209, R46211, H05016, H25436, AA236254, AA236301
791386	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2312 of SEQ ID NO:207, b is an integer of 15 to 2326, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:207, and where b is greater than or equal to a + 14.	
791598	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1448 of SEQ ID NO:208, b is an integer of 15 to 1462, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:208, and where b is greater than or equal to a + 14.	
791619	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2567 of SEQ ID NO:209, b is an integer of 15 to 2581, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:209, and where b is greater than or equal to a + 14.	R14767, R25924, R42537, R42537, R61122, R61844, H60027, H67016, W58641, W58640
791628	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1980 of SEQ ID NO:210, b is an integer of 15 to 1994, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:210, and where b is greater than or equal to a + 14.	
791751	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1500 of SEQ ID NO:211, b is an integer of 15 to 1514, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:211, and where b is greater than or equal to a + 14.	R09808, R68694, N32219, W63661, AA040449, AA234814, AA235276
792557	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 469 of SEQ ID NO:212, b is an integer of 15 to 483, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:212, and where b is greater than or equal to a + 14.	AA056147
792568	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 869 of SEQ ID NO:213, b is an integer of 15 to 883, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:213, and where b is greater than or equal to a + 14.	
792590	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 4785 of SEQ ID NO:214, b is an integer of 15 to 4799, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:214, and where b is greater than or equal to a + 14.	T64783, T72536, T80095, R13317, R18856, R24593, R40794, R44398, R44398, R40794, R75943, R76782, H84406, H84405, N26104, N26704, N34584, N36742, N36957, N46274, N48855, N53045, N67252, N73229, N75830, W07313, W38467, N90066, AA057494, AA187860, AA187859, AA253007, AA253130, AA258718, AA425229, AA425655
793323	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1031 of SEQ ID NO:215, b is an integer of 15 to 1045, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:215, and where b is greater than or equal to a + 14.	T55304, T58854, T61562, T90445, R07868, R07924, T66596, T78891, T82882, R15970, R32044, R32101, R56409, R64171, R64286, R71032, R71031, R77398, R77397, R79661, R79851, H26905, H47068, H47147, H47364, H48041, R92212, R92317, R95919, H50513, H51351, H52213, H52215, H57893, H57894, H61850, H79743, H79744, H82302, H85765, H94322, H94414, N20359, N25613, N26068, N34211, N35221, N40430, N54905, N62582, N69480, N70945, N74352, N74406, N75952, N76289, N80355, W02619, W04976, N90972, AA127903, AA459690, AA459811

793466	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1150 of SEQ ID NO:216, b is an integer of 15 to 1164, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:216, and where b is greater than or equal to a + 14.	
793507	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1580 of SEQ ID NO:217, b is an integer of 15 to 1594, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:217, and where b is greater than or equal to a + 14.	T68445, T68510, H11722, N54260, N64522, N80313, W74096, W79387, AA147027, AA426623, AA424798
793546	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1531 of SEQ ID NO:218, b is an integer of 15 to 1545, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:218, and where b is greater than or equal to a + 14.	
793559	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 448 of SEQ ID NO:219, b is an integer of 15 to 462, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:219, and where b is greater than or equal to a + 14.	
793604	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3080 of SEQ ID NO:220, b is an integer of 15 to 3094, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:220, and where b is greater than or equal to a + 14.	T64153, T64282, T94117, T94206, T87138, T81405, T81406, T85085, T86057, T97192, R01163, R06234, R14694, R14930, R32761, R32762, R41244, R42415, R52098, R52193, R41244, R42415, H10037, H10091, H11045, H11133, H24727, H24726, H24776, H24823, H26838, H44556, H44557, H61794, H61795, H83904, N28677, N32272, N37013, N40509, N46458, N57996, W51862, W73372, W73433, AA024892, AA024891, AA029877, AA029113, AA031341, AA036870, AA044325, AA044578, AA054735, AA054742, AA069699, AA084245, AA084244, AA120803, AA120804, AA227168, AA235731, AA459397, AA459622, AA464006, AA464713, AA425178, AA429092
794121	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1742 of SEQ ID NO:221, b is an integer of 15 to 1756, where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:221. and where b is greater than or equal to a + 14.	
794295	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 557 of SEQ ID NO:222. b is an integer of 15 to 571. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:222, and where b is greater than or equal to a + 14.	H62096. AA021403. AA224005
795241	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1683 of SEQ ID NO:223. b is an integer of 15 to 1697. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:223. and where b is greater than or equal to a + 14.	
795286	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2142 of SEQ ID NO:224. b is an integer of 15 to 2156. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:224, and where b is greater than or equal to a + 14.	T80215. T80216. R13602. R17713. R38783. R38784. R39897. R41783. R41783. R61528. R61584. H13658. H13659. H14690. H20561. H20654. H20770. H22585. R87081. R88769. R91028. R94865. R94866. N31866. N33177. N34225, N44964. N45304, N51118. N54239. N70835. W01441, W74260. W79873. W86917. W86947. W92091. AA010531. AA010532. AA011408. AA011464. AA130389, AA215587
795637	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1777 of SEQ ID NO:225. b is an integer of 15 to 1791, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:225, and where b is greater than or equal to a + 14.	
796301	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1511 of SEQ ID NO:226, b is an integer of 15 to 1525, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:226, and where b is greater than or equal to a + 14.	R05274, R86959. N55553, N76938. AA039578. AA042797. AA044610. AA243346. AA243547. AA262732, AA262814
796347	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1597 of SEQ ID NO:227, b is an integer of 15 to 1611, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:227. and where b is greater than or equal to a + 14.	
796579	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b.	T39155. T40439. T65119. T65188. R61110. R61832. H00285. H00286. H08348. H08349. N24725. N36706.

	where a is any integer between 1 to 1625 of SEQ ID NO:228. b is an integer of 15 to 1639. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:228. and where b is greater than or equal to a + 14.	N44806. N52179. N59471. N63112. N66486. N72051. W68534. W68821. W95493. W95530. AA055460. AA165066. AA164670. AA172036. AA172288. AA224152. AA256292. AA256434
796590	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1069 of SEQ ID NO:229. b is an integer of 15 to 1083. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:229. and where b is greater than or equal to a + 14.	
799783	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 345 of SEQ ID NO:230. b is an integer of 15 to 359. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:230. and where b is greater than or equal to a + 14.	
799784	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 341 of SEQ ID NO:231. b is an integer of 15 to 355. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:231, and where b is greater than or equal to a + 14.	
799785	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 360 of SEQ ID NO:232. b is an integer of 15 to 374. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:232. and where b is greater than or equal to a + 14.	
799786	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 418 of SEQ ID NO:233. b is an integer of 15 to 432. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:233. and where b is greater than or equal to a + 14.	
799787	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 352 of SEQ ID NO:234, b is an integer of 15 to 366, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:234. and where b is greater than or equal to a + 14.	
799800	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b.	

	where a is any integer between 1 to 414 of SEQ ID NO:235. b is an integer of 15 to 428. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:235. and where b is greater than or equal to a + 14.	
799808	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 952 of SEQ ID NO:236. b is an integer of 15 to 966. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:236. and where b is greater than or equal to a + 14.	W38424. W38440. W39289. W40123. W40239. W40423. W40223. W44752. W44840. W45263. W45310. W45466. W45478. W45484. W52088. W52399. W52587. W52966. W56192. W59966. W60273. W60443. W60621. W74243
799977	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 683 of SEQ ID NO:237. b is an integer of 15 to 697. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:237. and where b is greater than or equal to a + 14.	
800149	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2253 of SEQ ID NO:238. b is an integer of 15 to 2267. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:238. and where b is greater than or equal to a + 14.	
800189	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 753 of SEQ ID NO:239. b is an integer of 15 to 767. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:239. and where b is greater than or equal to a + 14.	
800589	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1704 of SEQ ID NO:240. b is an integer of 15 to 1718. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:240. and where b is greater than or equal to a + 14.	
800811	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 3585 of SEQ ID NO:241. b is an integer of 15 to 3599. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:241. and where b is greater than or equal to a + 14.	
800857	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2873 of SEQ ID	

	NO:242. b is an integer of 15 to 2887. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:242. and where b is greater than or equal to a + 14.	
805721	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1239 of SEQ ID NO:243. b is an integer of 15 to 1253. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:243. and where b is greater than or equal to a + 14.	
805818	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1588 of SEQ ID NO:244. b is an integer of 15 to 1602. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:244. and where b is greater than or equal to a + 14.	R37467. R43162. R49031. R43162. H90387. AA161488
806267	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1270 of SEQ ID NO:245. b is an integer of 15 to 1284. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:245. and where b is greater than or equal to a + 14.	
806579	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2080 of SEQ ID NO:246. b is an integer of 15 to 2094. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:246. and where b is greater than or equal to a + 14.	
810625	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1005 of SEQ ID NO:247. b is an integer of 15 to 1019. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:247. and where b is greater than or equal to a + 14.	
811153	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1486 of SEQ ID NO:248. b is an integer of 15 to 1500. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:248. and where b is greater than or equal to a + 14.	
811787	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2287 of SEQ ID NO:249. b is an integer of 15 to 2301. where both a	

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:249, and where b is greater than or equal to a + 14.	
812314	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2103 of SEQ ID NO:250, b is an integer of 15 to 2117, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:250, and where b is greater than or equal to a + 14.	
812443	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1432 of SEQ ID NO:251, b is an integer of 15 to 1446, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:251, and where b is greater than or equal to a + 14.	
812498	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2036 of SEQ ID NO:252, b is an integer of 15 to 2050, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:252, and where b is greater than or equal to a + 14.	
812504	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2515 of SEQ ID NO:253, b is an integer of 15 to 2529, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:253, and where b is greater than or equal to a + 14.	
813079	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1664 of SEQ ID NO:254, b is an integer of 15 to 1678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:254, and where b is greater than or equal to a + 14.	
815889	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 952 of SEQ ID NO:255, b is an integer of 15 to 966, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:255, and where b is greater than or equal to a + 14.	R75777, R81161, H89597, N66387, AA031510, AA031511, AA046590, AA046523, AA114840, AA114841, AA262053, AA459986, AA460079
824358	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3077 of SEQ ID NO:256, b is an integer of 15 to 3091, where both a and b correspond to the positions of nucleotide	

	residues shown in SEQ ID NO:256, and where b is greater than or equal to a + 14.	
826144	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2938 of SEQ ID NO:257, b is an integer of 15 to 2952, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:257, and where b is greater than or equal to a + 14.	T49872, R13469, R14630, R37379, R53048, R53135, R66676, R67394, R68165, R73097, R73098, H05459, H07010, H10504, H14581, H14671, H54297, H54374, H60845, H60931, H67688, H68011, N20226, N21171, N26851, N29134, N29294, N29562, N42173, AA026121, AA026205, AA136924, AA137020, AA460265, AA463830
826558	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2203 of SEQ ID NO:258, b is an integer of 15 to 2217, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:258, and where b is greater than or equal to a + 14.	T93500, R30805, R34197, R66925, R66924, H00931, H01734, H02282, H02385, W52225, AA040653, AA045530, AA058953, AA059458, AA127997, AA128093
827471	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1226 of SEQ ID NO:259, b is an integer of 15 to 1240, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:259, and where b is greater than or equal to a + 14.	
827716	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 596 of SEQ ID NO:260, b is an integer of 15 to 610, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:260, and where b is greater than or equal to a + 14.	
827722	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2102 of SEQ ID NO:261, b is an integer of 15 to 2116, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:261, and where b is greater than or equal to a + 14.	
827727	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1543 of SEQ ID NO:262, b is an integer of 15 to 1557, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:262, and where b is greater than or equal to a + 14.	
828238	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1640 of SEQ ID NO:263, b is an integer of 15 to 1654, where both a	AA193057, AA459842

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:263. and where b is greater than or equal to a + 14.	
828573	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1154 of SEQ ID NO:264. b is an integer of 15 to 1168. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:264. and where b is greater than or equal to a + 14.	W21349, AA287428. AA488879, AA736676. AA825689, AA831957
828624	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1743 of SEQ ID NO:265. b is an integer of 15 to 1757. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:265. and where b is greater than or equal to a + 14.	T80978, T80979. R63642. R63643. H50751. AA130349. AA130348, AA228511, AA229376. AA558367, AA588171. AA602572. AA902186. AA907305
828656	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 400 of SEQ ID NO:266. b is an integer of 15 to 414. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:266. and where b is greater than or equal to a + 14.	
828848	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1438 of SEQ ID NO:267. b is an integer of 15 to 1452, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:267. and where b is greater than or equal to a + 14.	W74302, C06154
828929	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 3045 of SEQ ID NO:268. b is an integer of 15 to 3059. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:268. and where b is greater than or equal to a + 14.	T71649. T66629. T82072. R16043. R18568, R25675, R27534, R37452, R37893. R49608, R49608, H00371, H04032. H15066, H15067, H17442, H25765. H25806, H42041, H42082, H98813. N21069, N26797. N27904, N30299. N32783, N35448. N39486, N41546. N42023, N47272, N48586, N51988, N53717, N62255. N72265, N95532, N95535, W02978. W24224, W24221. W37457, W49675. W49769, W94843. AA011118. AA017107, AA026474, AA026566, AA043220, AA053225. AA059038, AA127381, AA135518. AA135579, AA160002, AA161212. AA250957. AA251069, AA256560
829008	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 750 of SEQ ID NO:269. b is an integer of 15 to 764. where both a	

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:269, and where b is greater than or equal to a + 14.	
829086	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 518 of SEQ ID NO:270, b is an integer of 15 to 532, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:270, and where b is greater than or equal to a + 14.	
829192	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1383 of SEQ ID NO:271, b is an integer of 15 to 1397, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:271, and where b is greater than or equal to a + 14.	R01014, R18033, R68910, R99809, H52663, N58651, AA088731, AA193513, AA193662
829310	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 513 of SEQ ID NO:272, b is an integer of 15 to 527, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:272, and where b is greater than or equal to a + 14.	AA083295
829319	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 791 of SEQ ID NO:273, b is an integer of 15 to 805, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:273, and where b is greater than or equal to a + 14.	T90645, T90659, T97985, H06506, H19818, H20153, H20246, H21116, H21159, H21858, H41323, H41571, H42403, H42408, H42409, H42924, H42925, H44900, H46556, H50437, H50438, AA099620, AA102013, AA148703, AA148704
829459	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1939 of SEQ ID NO:274, b is an integer of 15 to 1953, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:274, and where b is greater than or equal to a + 14.	R09386, R09387, T78025, T97831, R23957, R23958, R24288, R24397, R26402, R28352, R28556, R28581, R63909, R63994, H02219, H04307, H04347, H06295, H06351, H13627, H13626, R84897, R85842, R98471, R98515, H72345, H81180, H95281, H95334, H99164, N29733, W03364, W47102, W47226, W92469, AA010223, AA011481, AA011482, AA016315, AA018837, AA101692, AA101805, AA101807, AA122274, AA121645, AA151559, AA149649, AA195694, AA195725, AA227519, AA232778, AA233860, AA234917, AA234918, AA253354, AA253355, AA258326, AA258535
829527	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2362 of SEQ ID NO:275, b is an integer of 15 to 2376, where both a	T58131, T63068, T90761, T80172, T83210, T96126, T96208, R02011, R02010, R13993, R37587, R39116, R49772, H04979, H04978, H10390, H10599, H25348, R89064, R89161,

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:275. and where b is greater than or equal to a + 14.	W40553. W42765. W57719. W57718, AA125861. AA125860. AA187443. AA187617. AA234055. AA430020
829736	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2425 of SEQ ID NO:276. b is an integer of 15 to 2439. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:276, and where b is greater than or equal to a + 14.	T49267, T49268, T49304, T49305. T63879. T80451. T81311. T81839. T83362. T83508. T95341. T95436. R22333, R25604. R34248. R35407. R35574. R49204. R49204. R62803, R62852. H13144. H17521. H44982, R93505. R93504, H98806. N24673, N25026. N32953. N33048. N35464, N42110. N42625. N55468. N76843. W03837. AA056568. AA056719. AA150946. AA151038. AA165138, AA169548. AA169352. AA171757, AA171713. AA171996. AA172106. AA235604. AA424478
830552	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1875 of SEQ ID NO:277. b is an integer of 15 to 1889. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:277. and where b is greater than or equal to a + 14.	
830566	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 622 of SEQ ID NO:278. b is an integer of 15 to 636. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:278, and where b is greater than or equal to a + 14.	H58586
830568	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2847 of SEQ ID NO:279. b is an integer of 15 to 2861, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:279, and where b is greater than or equal to a + 14.	T86173, T86174. R31229. R56392. H27334, H41900, H41939. N41528. AA464551. AA464652. AA425346. AA430320. AA514778. AA551699, AA558620. AA558725. AA583577. AA612719. AA574033. AA746483. AA808281. AA831559. AA873069, AA878486, W22260, W22881, N88548, C04008. C04877. C05565
830569	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1492 of SEQ ID NO:280, b is an integer of 15 to 1506. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:280. and where b is greater than or equal to a + 14.	AA148863. AA148864
830583	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1679 of SEQ ID NO:281. b is an integer of 15 to 1693. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:281. and where b is	

	greater than or equal to a + 14.	
830613	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1209 of SEQ ID NO:282, b is an integer of 15 to 1223, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:282, and where b is greater than or equal to a + 14.	
830686	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 476 of SEQ ID NO:283, b is an integer of 15 to 490, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:283, and where b is greater than or equal to a + 14.	
830691	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2995 of SEQ ID NO:284, b is an integer of 15 to 3009, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:284, and where b is greater than or equal to a + 14.	T64847, T72590, R21403, R46500, R46500, R59229, R59289, H30531, H40605, H46249, H46370, H49841, H91758, AA125799, AA135387, AA135994, AA464935, AA424273, AA568294, AA810246, D80751, D81702, AA092153
830716	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 862 of SEQ ID NO:285, b is an integer of 15 to 876, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:285, and where b is greater than or equal to a + 14.	
830792	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 847 of SEQ ID NO:286, b is an integer of 15 to 861, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:286, and where b is greater than or equal to a + 14.	
830893	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1054 of SEQ ID NO:287, b is an integer of 15 to 1068, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:287, and where b is greater than or equal to a + 14.	
830976	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2242 of SEQ ID NO:288, b is an integer of 15 to 2256, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:288, and where b is greater than or equal to a + 14.	

831043	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 317 of SEQ ID NO:289, b is an integer of 15 to 331, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:289, and where b is greater than or equal to a + 14.	
831131	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 691 of SEQ ID NO:290, b is an integer of 15 to 705, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:290, and where b is greater than or equal to a + 14.	
831164	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 938 of SEQ ID NO:291, b is an integer of 15 to 952, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:291, and where b is greater than or equal to a + 14.	T74499, R12051, R18399, R60836, H15297, H18858, H23172, AA721309, AA831174, C04626
831173	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 590 of SEQ ID NO:292, b is an integer of 15 to 604, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:292, and where b is greater than or equal to a + 14.	
831255	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 496 of SEQ ID NO:293, b is an integer of 15 to 510, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:293, and where b is greater than or equal to a + 14.	
831327	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 831 of SEQ ID NO:294, b is an integer of 15 to 845, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:294, and where b is greater than or equal to a + 14.	W38432, W44821, W51893, W51781, W52725, W59978, W60116, AA588704, C05911, C05915
831493	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1032 of SEQ ID NO:295, b is an integer of 15 to 1046, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:295, and where b is greater than or equal to a + 14.	
831500	Preferably excluded from the present invention are	T67004, T67005, R06266, R06324,

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1902 of SEQ ID NO:296, b is an integer of 15 to 1916, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:296, and where b is greater than or equal to a + 14.	R55532, R55533, W60669, W60670, W96122, W96123, AA551364, AA553611, AA570432
831501	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1462 of SEQ ID NO:297, b is an integer of 15 to 1476, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:297, and where b is greater than or equal to a + 14.	R52091, H14837, AA023003, AA022470, AA232097, AA256032, AA258844, AA259023, AA424828, AA557330, AA765793
831502	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 527 of SEQ ID NO:298, b is an integer of 15 to 541, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:298, and where b is greater than or equal to a + 14.	
831508	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 457 of SEQ ID NO:299, b is an integer of 15 to 471, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:299, and where b is greater than or equal to a + 14.	
831509	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 928 of SEQ ID NO:300, b is an integer of 15 to 942, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:300, and where b is greater than or equal to a + 14.	
831520	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 447 of SEQ ID NO:301, b is an integer of 15 to 461, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:301, and where b is greater than or equal to a + 14.	
831547	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 892 of SEQ ID NO:302, b is an integer of 15 to 906, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:302, and where b is greater than or equal to a + 14.	R09826, T95977, T97888, H66377, W31141
831548	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide	T95880, T97781, R05685, R12413, R37130, R37412, R94523, H82826,

	sequence described by the general formula of a-b, where a is any integer between 1 to 606 of SEQ ID NO:303, b is an integer of 15 to 620, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:303, and where b is greater than or equal to a + 14.	H99806, H99813, AA172251, AA468699, AA659754, AA808925, AA837298, AA858110, AA864723, AA954263, F18115, N99864
831558	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 519 of SEQ ID NO:304, b is an integer of 15 to 533, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:304, and where b is greater than or equal to a + 14.	H60157, W57916, W57917, AA056029, AA056047, AA142858, AA211887, AA469104, AA659257, AA662867, AA665372, AA728846, AA933045, F17890, AA090265
831847	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1360 of SEQ ID NO:305, b is an integer of 15 to 1374, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:305, and where b is greater than or equal to a + 14.	
831893	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 654 of SEQ ID NO:306, b is an integer of 15 to 668, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:306, and where b is greater than or equal to a + 14.	
831903	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1032 of SEQ ID NO:307, b is an integer of 15 to 1046, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:307, and where b is greater than or equal to a + 14.	
831921	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1672 of SEQ ID NO:308, b is an integer of 15 to 1686, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:308, and where b is greater than or equal to a + 14.	H52554, H66743, H71667, N32238, N77727, W19857, AA017111, AA074918, AA235917, AA236708
831923	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1412 of SEQ ID NO:309, b is an integer of 15 to 1426, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:309, and where b is greater than or equal to a + 14.	
831959	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b,	

	where a is any integer between 1 to 1479 of SEQ ID NO:310. b is an integer of 15 to 1493. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:310, and where b is greater than or equal to a + 14.	
832008	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 2328 of SEQ ID NO:311. b is an integer of 15 to 2342, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:311. and where b is greater than or equal to a + 14.	
832107	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 840 of SEQ ID NO:312. b is an integer of 15 to 854. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:312. and where b is greater than or equal to a + 14.	N38762. W81128. W81129
832110	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1487 of SEQ ID NO:313, b is an integer of 15 to 1501. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:313. and where b is greater than or equal to a + 14.	W72867. W76102. AA557708
832146	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1179 of SEQ ID NO:314. b is an integer of 15 to 1193. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:314. and where b is greater than or equal to a + 14.	
832189	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 784 of SEQ ID NO:315. b is an integer of 15 to 798. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:315. and where b is greater than or equal to a + 14.	AA004742. AA236306
832295	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1921 of SEQ ID NO:316. b is an integer of 15 to 1935, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:316, and where b is greater than or equal to a + 14.	H21746. H21943, H39580. AA455263. AA455264, AA465644, AA563903. AA576922. AA661801. AA747311. AA767674, AA933667, AI088750
832334	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1724 of SEQ ID	

	NO:317. b is an integer of 15 to 1738, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:317, and where b is greater than or equal to a + 14.	
832339	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1326 of SEQ ID NO:318. b is an integer of 15 to 1340. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:318, and where b is greater than or equal to a + 14.	
832393	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 770 of SEQ ID NO:319. b is an integer of 15 to 784. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:319. and where b is greater than or equal to a + 14.	
832415	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 3513 of SEQ ID NO:320. b is an integer of 15 to 3527. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:320, and where b is greater than or equal to a + 14.	T65740, R78913, R79012, R82303, R82302, H13769, H81248, H81589, H88099, H95138, H97042, H81589, N21407, N25252, N29919, N31363, N33888, N42972, N50375, N51590, W38583, W69205, W69309, W73506, W73337, N90198, AA099534, AA099533, AA173671, AA173689, AA252476
832422	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1435 of SEQ ID NO:321, b is an integer of 15 to 1449. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:321, and where b is greater than or equal to a + 14.	T99380, T99603, N31610, N32587, N42671, N47813, AA009818, AA009819, AA166785, AA166950, AA507182, AA569843, D78758, C04932
832448	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 763 of SEQ ID NO:322. b is an integer of 15 to 777, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:322, and where b is greater than or equal to a + 14.	
832532	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1200 of SEQ ID NO:323, b is an integer of 15 to 1214, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:323. and where b is greater than or equal to a + 14.	
832621	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1032 of SEQ ID	W24985, W47319, AA922747

	NO:324. b is an integer of 15 to 1046, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:324, and where b is greater than or equal to a + 14.	
832622	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 660 of SEQ ID NO:325, b is an integer of 15 to 674, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:325, and where b is greater than or equal to a + 14.	
835327	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 343 of SEQ ID NO:326, b is an integer of 15 to 357, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:326, and where b is greater than or equal to a + 14.	
835695	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1565 of SEQ ID NO:327, b is an integer of 15 to 1579, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:327, and where b is greater than or equal to a + 14.	
835857	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2258 of SEQ ID NO:328, b is an integer of 15 to 2272, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:328, and where b is greater than or equal to a + 14.	
836183	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1306 of SEQ ID NO:329, b is an integer of 15 to 1320, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:329, and where b is greater than or equal to a + 14.	
836190	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1846 of SEQ ID NO:330, b is an integer of 15 to 1860, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:330, and where b is greater than or equal to a + 14.	
836196	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1562 of SEQ ID NO:331. b is an integer of 15 to 1576, where both a	T53851, T53923, R63103, R76448, R76703, N35338, N44709, N75001, N98466, N98613, N98769, W05702, W24237, W31023, W30985, W38813, W38941, W42920, W42850, W47106,

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:331. and where b is greater than or equal to a + 14.	W47230. W56833. W60274, W67278. W67414. N89826. AA043314. AA043313. AA046060, AA046186, AA102070. AA099937. AA502040. AA507883. AA507901. AA533422. AA847757. AA877285. AA878535. AA887648. AA970407. AA653954. AA291528
836253	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 562 of SEQ ID NO:332. b is an integer of 15 to 576. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:332, and where b is greater than or equal to a + 14.	
836372	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1297 of SEQ ID NO:333. b is an integer of 15 to 1311. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:333. and where b is greater than or equal to a + 14.	
837077	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1104 of SEQ ID NO:334, b is an integer of 15 to 1118, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:334. and where b is greater than or equal to a + 14.	AA604913. AA576835. AA862767, AA902805. AI080476
837445	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2252 of SEQ ID NO:335, b is an integer of 15 to 2266. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:335, and where b is greater than or equal to a + 14.	
837620	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1118 of SEQ ID NO:336, b is an integer of 15 to 1132. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:336. and where b is greater than or equal to a + 14.	
837981	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2215 of SEQ ID NO:337, b is an integer of 15 to 2229, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:337. and where b is greater than or equal to a + 14.	
837995	Preferably excluded from the present invention are	T51581. T68704. T68747. T68770.

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3714 of SEQ ID NO:338, b is an integer of 15 to 3728, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:338, and where b is greater than or equal to a + 14.	T68795, T68814, T73080, T73178, T73508, T83922, T87588, T78456, T78483, T78523, T78568, T79931, T83750, R16916, R16973, R73535, R73536, R95125, R95126, R99128, H48427, H65045, H65046, H65601, H72506, H72904, H73672, H73416, H75352, H79656, N55345, N69659, N77351, N94268, N94637, W19274, W23857, W24361, W42977, W48819, W68303, W68486, AA037188, AA044094, AA044284, AA055252, AA055253, AA186602, AA188281, AA177045, AA229943, AA514508, AA557392, AA565513, H80617, AA588181, AA635650, AA580469, AA687441, AA687497, AA834363, AA878670, AA906758, AA934579, AA948660, AA995311, C06397, AA284956, AA285113, AA292550
838001	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2660 of SEQ ID NO:339, b is an integer of 15 to 2674, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:339, and where b is greater than or equal to a + 14.	
838237	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1443 of SEQ ID NO:340, b is an integer of 15 to 1457, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:340, and where b is greater than or equal to a + 14.	
838700	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3385 of SEQ ID NO:341, b is an integer of 15 to 3399, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:341, and where b is greater than or equal to a + 14.	
838805	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1915 of SEQ ID NO:342, b is an integer of 15 to 1929, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:342, and where b is greater than or equal to a + 14.	
839096	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1547 of SEQ ID NO:343, b is an integer of 15 to 1561, where both a	

	and b correspond to the positions of nucleotide residues shown in SEQ ID NO:343. and where b is greater than or equal to a + 14.	
839185	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2968 of SEQ ID NO:344. b is an integer of 15 to 2982. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:344. and where b is greater than or equal to a + 14.	
839588	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1640 of SEQ ID NO:345. b is an integer of 15 to 1654. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:345. and where b is greater than or equal to a + 14.	
839589	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 484 of SEQ ID NO:346. b is an integer of 15 to 498. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:346. and where b is greater than or equal to a + 14.	
839733	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3162 of SEQ ID NO:347. b is an integer of 15 to 3176. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:347. and where b is greater than or equal to a + 14.	T49124. T49125, T87606, T80183, R17716. R25789, R37588, R41786, R46788. R41786. R46788, R86012, N27045. N27365, N31477. N75044, N80842. N92937, N99972, W05771, AA007622, AA007661, AA035367, AA135176. AA135350. AA458470, AA505865, AA506506. AA526375, AA613311. AA613813. AA636046, AA639686, AA569896. AA687824, AA740795, AA828494, AA830137, AA836424, AA902192. AA907444, AA910103. AA916663. AA961769, AA987257, AA995286, C02440, C03271. C04496, AA400614, AA401259, AA401972. AA402117, AA404233, AA442982, AA453509, AA453510, AA454684, AA456333, AA845142, AA854089. AA813552, AA860919, AI024368. AI078067. D30835. D31579
839874	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1113 of SEQ ID NO:348. b is an integer of 15 to 1127, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:348, and where b is greater than or equal to a + 14.	H11826, H19387, AA082620
840017	Preferably excluded from the present invention are	

	one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2121 of SEQ ID NO:349, b is an integer of 15 to 2135, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:349, and where b is greater than or equal to a + 14.	
840124	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1564 of SEQ ID NO:350, b is an integer of 15 to 1578, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:350, and where b is greater than or equal to a + 14.	
840222	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 960 of SEQ ID NO:351, b is an integer of 15 to 974, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:351, and where b is greater than or equal to a + 14.	R84486, R84529, R88248, Z43097
840617	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2587 of SEQ ID NO:352, b is an integer of 15 to 2601, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:352, and where b is greater than or equal to a + 14.	
840641	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 907 of SEQ ID NO:353, b is an integer of 15 to 921, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:353, and where b is greater than or equal to a + 14.	H50311, N31637, N38837, N57092, W25229, W35251, W58039, W58123, W72521, W76080, N89999, AA256075, AA256114, AA426416, AA279475, AA287965, AA286961, AA286962, AA405003, AA521338, AA588308, AA729660, AA732508, AA736855, AA760789, AA765636, AA766365, AA805546, AA825927, AA911323, AA917840, AA918945, AA922719, AA939023, AA969474, AA976724, N95393, AA453687, AA482391, AA447756, AA706719, AA709036, AA719892, AI089099, D20399
840792	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1297 of SEQ ID NO:354, b is an integer of 15 to 1311, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:354, and where b is greater than or equal to a + 14.	R23893, R23892, R32223, R81610, H00321, N30960, N66394, W40278, W40275, W45359, W56625, W56539, AA025789, AA025949, AA126511, AA126636, AA131184, AA131120, AA131260, AA135445, AA164894, AA164893, AA181943, AA262234, AA460727, AA460899, AA614654, AA576166, AA577101, AA577111, AA814470, AA962227, AA996044, C00083, C18672, AA644060, AA635144, AA725839, AA960853,

		AA992056. AI003313. AI014315. AI024320. AI122746. T24622
840915	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2239 of SEQ ID NO:355. b is an integer of 15 to 2253. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:355. and where b is greater than or equal to a + 14.	
841059	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1221 of SEQ ID NO:356. b is an integer of 15 to 1235. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:356. and where b is greater than or equal to a + 14.	
841325	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1394 of SEQ ID NO:357. b is an integer of 15 to 1408. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:357, and where b is greater than or equal to a + 14.	R28417. R28429. AA279887. AA481504
841713	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 858 of SEQ ID NO:358. b is an integer of 15 to 872. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:358, and where b is greater than or equal to a + 14.	
842324	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1730 of SEQ ID NO:359, b is an integer of 15 to 1744. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:359. and where b is greater than or equal to a + 14.	
842386	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 659 of SEQ ID NO:360. b is an integer of 15 to 673. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:360. and where b is greater than or equal to a + 14.	
842454	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b. where a is any integer between 1 to 1310 of SEQ ID NO:361. b is an integer of 15 to 1324. where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:361. and where b is	

	greater than or equal to a + 14.	
842768	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 664 of SEQ ID NO:362, b is an integer of 15 to 678, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:362, and where b is greater than or equal to a + 14.	
842999	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 5222 of SEQ ID NO:363, b is an integer of 15 to 5236, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:363, and where b is greater than or equal to a + 14.	
843830	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1006 of SEQ ID NO:364, b is an integer of 15 to 1020, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:364, and where b is greater than or equal to a + 14.	
844723	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2194 of SEQ ID NO:365, b is an integer of 15 to 2208, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:365, and where b is greater than or equal to a + 14.	
844868	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2741 of SEQ ID NO:366, b is an integer of 15 to 2755, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:366, and where b is greater than or equal to a + 14.	
845258	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 1950 of SEQ ID NO:367, b is an integer of 15 to 1964, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:367, and where b is greater than or equal to a + 14.	R24215, R24216, R66047, R66048, H02011, H12618, H12668, H90748, H90799, N69833, N93931, N98972, W40431, W90007, AA024872, AA115390, AA133417, AA194946, AA195087, AA195556, AA195715, AA195752, AA425375, AA425467, AA903701, AI078393, Z44587, AA700297, AA702853
845373	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 3033 of SEQ ID NO:368, b is an integer of 15 to 3047, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:368, and where b is	

	greater than or equal to a + 14.	
845412	Preferably excluded from the present invention are one or more polynucleotides comprising a nucleotide sequence described by the general formula of a-b, where a is any integer between 1 to 2397 of SEQ ID NO:369, b is an integer of 15 to 2411, where both a and b correspond to the positions of nucleotide residues shown in SEQ ID NO:369, and where b is greater than or equal to a + 14.	

Polynucleotide and Polypeptide Variants

The present invention is directed to variants of the polynucleotide sequence disclosed in SEQ ID NO:X or the complementary strand thereto, and/or the cDNA sequence contained in a cDNA clone contained in the deposit.

The present invention also encompasses variants of the pancreas and pancreatic cancer polypeptide sequence disclosed in SEQ ID NO:Y, a polypeptide sequence encoded by the polynucleotide sequence in SEQ ID NO:X, and/or a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

"Variant" refers to a polynucleotide or polypeptide differing from the polynucleotide or polypeptide of the present invention, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the polynucleotide or polypeptide of the present invention.

The present invention is also directed to nucleic acid molecules which comprise, or alternatively consist of, a nucleotide sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the nucleotide coding sequence in SEQ ID NO:X or the complementary strand thereto, the nucleotide coding sequence of the related cDNA contained in a deposited library or the complementary strand thereto, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polypeptides encoded by these nucleic acid molecules are also encompassed by the invention. In another embodiment, the invention encompasses nucleic acid molecules which comprise or alternatively consist of, a polynucleotide which hybridizes under stringent hybridization conditions, or alternatively, under low stringency conditions, to the nucleotide coding sequence in SEQ ID NO:X, the nucleotide coding sequence of the related cDNA clone contained in a deposited library, a nucleotide sequence encoding the polypeptide of SEQ ID NO:Y, a nucleotide sequence encoding a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a nucleotide sequence encoding the polypeptide encoded by the cDNA in the related cDNA clone contained in a deposited library, and/or polynucleotide fragments of any of these nucleic acid molecules (e.g., those fragments described herein). Polynucleotides which

hybridize to the complement of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

The present invention is also directed to polypeptides which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to, for example, the polypeptide sequence shown in SEQ ID NO:Y, a polypeptide sequence encoded by the nucleotide sequence in SEQ ID NO:X, a polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library, and/or polypeptide fragments of any of these polypeptides (e.g., those fragments described herein). Polynucleotides which hybridize to the complement of the nucleic acid molecules encoding these polypeptides under stringent hybridization conditions, or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides.

By a nucleic acid having a nucleotide sequence at least, for example, 95% "identical" to a reference nucleotide sequence of the present invention, it is intended that the nucleotide sequence of the nucleic acid is identical to the reference sequence except that the nucleotide sequence may include up to five point mutations per each 100 nucleotides of the reference nucleotide sequence encoding the polypeptide. In other words, to obtain a nucleic acid having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence. The query sequence may be, for example, an entire sequence referred to in Table 1, an ORF (open reading frame), or any fragment specified as described herein.

As a practical matter, whether any particular nucleic acid molecule or polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a nucleotide sequence of the present invention can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci. 6:237-245 (1990)). In a sequence alignment the query and subject sequences are both DNA sequences. An RNA sequence can be

compared by converting U's to T's. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB alignment of DNA sequences to calculate percent identiy are: Matrix=Unitary, k-tuple=4, Mismatch Penalty=1, Joining Penalty=30, Randomization Group Length=0, Cutoff Score=1, Gap Penalty=5, Gap Size Penalty 0.05, Window Size=500 or the lenght of the subject nucleotide sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence because of 5' or 3' deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for 5' and 3' truncations of the subject sequence when calculating percent identity. For subject sequences truncated at the 5' or 3' ends, relative to the query sequence, the percent identity is corrected by calculating the number of bases of the query sequence that are 5' and 3' of the subject sequence, which are not matched/aligned, as a percent of the total bases of the query sequence. Whether a nucleotide is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This corrected score is what is used for the purposes of the present invention. Only bases outside the 5' and 3' bases of the subject sequence, as displayed by the FASTDB alignment, which are not matched/aligned with the query sequence, are calculated for the purposes of manually adjusting the percent identity score.

For example, a 90 base subject sequence is aligned to a 100 base query sequence to determine percent identity. The deletions occur at the 5' end of the subject sequence and therefore, the FASTDB alignment does not show a matched/alignment of the first 10 bases at 5' end. The 10 unpaired bases represent 10% of the sequence (number of bases at the 5' and 3' ends not matched/total number of bases in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 bases were perfectly matched the final percent identity would be 90%. In another example, a 90 base subject sequence is compared with a 100 base query sequence. This time the deletions are internal deletions so that there are no bases on the 5' or 3' of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only bases 5' and 3' of the subject sequence which are not matched/aligned with the query sequence are manually corrected for. No other

manual corrections are to made for the purposes of the present invention.

By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, (indels) or substituted with another amino acid. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence in SEQ ID NO:Y or a fragment thereof, the amino acid sequence encoded by the nucleotide sequence in SEQ ID NO:X or a fragment thereof, or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library, or a fragment thereof, can be determined conventionally using known computer programs. A preferred method for determing the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237- 245(1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is in percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences

truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to made for the purposes of the present invention.

The variants may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, variants in which less than 50, less

than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host such as E. coli).

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985).) These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, as discussed herein, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. The authors of Ron et al., J. Biol. Chem. 268: 2984-2988 (1993), reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." (See, Abstract.) In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

Furthermore, as discussed herein, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more

biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

Thus, the invention further includes polypeptide variants which show a functional activity (e.g., biological activity) of the polypeptide of the invention of which they are a variant. Such variants include deletions, insertions, inversions, repeats, and substitutions selected according to general rules known in the art so as have little effect on activity.

The present application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein or fragments thereof, (e.g., including but not limited to fragments encoding a polypeptide having the amino acid sequence of an N and/or C terminal deletion), irrespective of whether they encode a polypeptide having functional activity. This is because even where a particular nucleic acid molecule does not encode a polypeptide having functional activity, one of skill in the art would still know how to use the nucleic acid molecule, for instance, as a hybridization probe or a polymerase chain reaction (PCR) primer. Uses of the nucleic acid molecules of the present invention that do not encode a polypeptide having functional activity include, inter alia, (1) isolating a gene or allelic or splice variants thereof in a cDNA library; (2) in situ hybridization (e.g., "FISH") to metaphase chromosomal spreads to provide precise chromosomal location of the gene, as described in Verma et al., Human Chromosomes: A Manual of Basic Techniques, Pergamon Press, New York (1988); and (3) Northern Blot analysis for detecting mRNA expression in specific tissues.

Preferred, however, are nucleic acid molecules having sequences at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% identical to the nucleic acid sequences disclosed herein, which do, in fact, encode a polypeptide having a functional activity of a polypeptide of the invention.

Of course, due to the degeneracy of the genetic code, one of ordinary skill in the art will immediately recognize that a large number of the nucleic acid molecules having a sequence at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identical to, for example, the nucleic acid sequence of the cDNA in the related cDNA clone contained in a

deposited library, the nucleic acid sequence referred to in Table 1 (SEQ ID NO:X), or fragments thereof. will encode polypeptides "having functional activity." In fact, since degenerate variants of any of these nucleotide sequences all encode the same polypeptide, in many instances, this will be clear to the skilled artisan even without performing the above described comparison assay. It will be further recognized in the art that, for such nucleic acid molecules that are not degenerate variants, a reasonable number will also encode a polypeptide having functional activity. This is because the skilled artisan is fully aware of amino acid substitutions that are either less likely or not likely to significantly effect protein function (e.g., replacing one aliphatic amino acid with a second aliphatic amino acid), as further described below.

For example, guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," Science 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. (Cunningham and Wells, Science 244:1081-1085 (1989).) The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side

chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) substitutions with one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) substitution with one or more of amino acid residues having a substituent group, or (iii) fusion of the mature polypeptide with another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), or (iv) fusion of the polypeptide with additional amino acids, such as, for example, an IgG Fc fusion region peptide, or leader or secretory sequence, or a sequence facilitating purification. Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. (Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).)

A further embodiment of the invention relates to a polypeptide which comprises the amino acid sequence of a polypeptide having an amino acid sequence which contains at least one amino acid substitution, but not more than 50 amino acid substitutions, even more preferably, not more than 40 amino acid substitutions, still more preferably, not more than 30 amino acid substitutions, and still even more preferably, not more than 20 amino acid substitutions. Of course it is highly preferable for a polypeptide to have an amino acid sequence which comprises the amino acid sequence of a polypeptide of SEQ ID NO:Y, an amino acid sequence encoded by SEQ ID NO:X, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library which contains, in order of ever-increasing preference, at least one, but not more than 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1

amino acid substitutions. In specific embodiments, the number of additions, substitutions, and/or deletions in the amino acid sequence of SEQ ID NO:Y or fragments thereof (e.g., the mature form and/or other fragments described herein), an amino acid sequence encoded by SEQ ID NO:X or fragments thereof, and/or the amino acid sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or fragments thereof, is 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, conservative amino acid substitutions are preferable.

Polynucleotide and Polypeptide Fragments

The present invention is also directed to polynucleotide fragments of the pancreas and pancreatic cancer polynucleotides (nucleic acids) of the invention. In the present invention, a "polynucleotide fragment" refers, for example, to a polynucleotide having a nucleic acid sequence which: is a portion of the cDNA contained in a depostied cDNA clone; or is a portion of a polynucleotide sequence encoding the polypeptide encoded by the cDNA contained in a deposited cDNA clone; or is a portion of the polynucleotide sequence in SEQ ID NO:X or the complementary strand thereto; or is a polynucleotide sequence encoding a portion of the polypeptide of SEQ ID NO:Y; or is a polynucleotide sequence encoding a portion of a polypeptide encoded by SEQ ID NO:X or the complementary strand thereto. The nucleotide fragments of the invention are preferably at least about 15 nt, and more preferably at least about 20 nt, still more preferably at least about 30 nt, and even more preferably, at least about 40 nt, at least about 50 nt, at least about 75 nt, at least about 100 nt, at least about 125 nt or at least about 150 nt in length. A fragment "at least 20 nt in length," for example, is intended to include 20 or more contiguous bases from, for example, the sequence contained in the cDNA in a related cDNA clone contained in a deposited library, the nucleotide sequence shown in SEQ ID NO:X or the complementary stand thereto. In this context "about" includes the particularly recited value or a value larger or smaller by several (5, 4, 3, 2, or 1) nucleotides. These nucleotide fragments have uses that include, but are not limited to, as diagnostic probes and primers as discussed herein. Of course, larger fragments (e.g., at least 150, 175, 200, 250, 500, 600, 1000, or 2000 nucleotides in length) are also encompassed by the invention.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-

400, 401-450, 451-500, 501-550, 551-600, 651-700,701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of SEQ ID NO:X, or the complementary strand thereto. In this context "about" includes the particularly recited range or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a functional activity (e.g., biological activity) of the polypeptide encoded by the polynucleotide of which the sequence is a portion. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these nucleic acid molecules under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

Moreover, representative examples of polynucleotide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, a sequence from about nucleotide number 1-50, 51-100, 101-150, 151-200, 201-250, 251-300, 301-350, 351-400, 401-450, 451-500, 501-550, 551-600, 651-700,701- 750, 751-800, 800-850, 851-900, 901-950, 951-1000, 1001-1050, 1051-1100, 1101-1150, 1151-1200, 1201-1250, 1251-1300, 1301-1350, 1351-1400, 1401-1450, 1451-1500, 1501-1550, 1551-1600, 1601-1650, 1651-1700, 1701-1750, 1751-1800, 1801-1850, 1851-1900, 1901-1950, 1951-2000, 2001-2050, 2051-2100, 2101-2150, 2151-2200, 2201-2250, 2251-2300, 2301-2350, 2351-2400, 2401-2450, 2451-2500, 2501-2550, 2551-2600, 2601-2650, 2651-2700, 2701-2750, 2751-2800, 2801-2850, 2851-2900, 2901-2950, 2951-3000, 3001-3050, 3051-3100, 3101-3150, 3151-3200, 3201-3250, 3251-3300, 3301-3350, 3351-3400, 3401-3450, 3451-3500, 3501-3550, and 3551 to the end of the cDNA nucleotide sequence contained in the deposited cDNA clone, or the complementary strand thereto. In this context "about" includes the particularly recited range, or a range larger or smaller by several (5, 4, 3, 2, or 1) nucleotides, at either terminus or at both termini. Preferably, these fragments encode a polypeptide which has a

functional activity (e.g., biological activity) of the polypeptide encoded by the cDNA nucleotide sequence contained in the deposited cDNA clone. More preferably, these fragments can be used as probes or primers as discussed herein. Polynucleotides which hybridize to one or more of these fragments under stringent hybridization conditions or alternatively, under lower stringency conditions, are also encompassed by the invention, as are polypeptides encoded by these polynucleotides or fragments.

In the present invention, a "polypeptide fragment" refers to an amino acid sequence which is a portion of that contained in SEQ ID NO:Y, a portion of an amino acid sequence encoded by the polynucleotide sequence of SEQ ID NO:X, and/or encoded by the cDNA contained in the related cDNA clone contained in a deposited library. Protein (polypeptide) fragments may be "free-standing," or comprised within a larger polypeptide of which the fragment forms a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments comprising, or alternatively consisting of, an amino acid sequence from about amino acid number 1-20, 21-40, 41-60, 61-80, 81-100, 102-120, 121-140, 141-160, 161-180, 181-200, 201-220, 221-240, 241-260, 261-280, 281-300, 301-320, 321-340, 341-360, 361-380, 381-400, 401-420, 421-440, 441-460, 461-480, 481-500, 501-520, 521-540, 541-560, 561-580, 581-600, 601-620, 621-640, 641-660, 661-680, 681-700, 701-720, 721-740, 741-760, 761-780, 781-800, 801-820, 821-840, 841-860, 861-880, 881-900, 901-920, 921-940, 941-960, 961-980, 981-1000, 1001-1020, 1021-1040, 1041-1060, 1061-1080, 1081-1100, 1101-1120, 1121-1140, 1141-1160, 1161-1180, and 1181 to the end of SEQ ID NO:Y. Moreover, polypeptide fragments of the invention may be at least about 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 100, 110, 120, 130, 140, or 150 amino acids in length. In this context "about" includes the particularly recited ranges or values, or ranges or values larger or smaller by several (5, 4, 3, 2, or 1) amino acids, at either terminus or at both termini. Polynucleotides encoding these polypeptide fragments are also encompassed by the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of shortened muteins to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained

when less than the majority of the residues of the complete or mature polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, polypeptide fragments of the invention include the secreted protein as well as the mature form. Further preferred polypeptide fragments include the secreted protein or the mature form having a continuous series of deleted residues from the amino or the carboxy terminus, or both. For example, any number of amino acids, ranging from 1-60, can be deleted from the amino terminus of either the secreted polypeptide or the mature form. Similarly, any number of amino acids, ranging from 1-30, can be deleted from the carboxy terminus of the secreted protein or mature form. Furthermore, any combination of the above amino and carboxy terminus deletions are preferred. Similarly, polynucleotides encoding these polypeptide fragments are also preferred.

The present invention further provides polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in the related cDNA clone contained in a deposited library). In particular, N-terminal deletions may be described by the general formula m-q, where q is a whole integer representing the total number of amino acid residues in a polypeptide of the invention (e.g., the polypeptide disclosed in SEQ ID NO:Y), and m is defined as any integer ranging from 2 to q-6. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Also as mentioned above, even if deletion of one or more amino acids from the C-terminus of a protein results in modification of loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example the ability of the shortened mutein to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular

polypeptide lacking C-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted C-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, the present invention further provides polypeptides having one or more residues from the carboxy terminus of the amino acid sequence of a polypeptide disclosed herein (e.g., a polypeptide of SEQ ID NO:Y, a polypeptide encoded by the polynucleotide sequence contained in SEQ ID NO:X, and/or a polypeptide encoded by the cDNA contained in deposited cDNA clone referenced in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where n corresponds to the position of an amino acid residue in a polypeptide of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a polypeptide encoded by SEQ ID NO:X (e.g., including, but not limited to, the preferred polypeptide disclosed as SEQ ID NO:Y), and/or the cDNA in the related cDNA clone contained in a deposited library, where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Any polypeptide sequence contained in the polypeptide of SEQ ID NO:Y, encoded by the polynucleotide sequences set forth as SEQ ID NO:X, or encoded by the cDNA in the related cDNA clone contained in a deposited library may be analyzed to determine certain preferred regions of the polypeptide. For example, the amino acid sequence of a polypeptide encoded by a polynucleotide sequence of SEQ ID NO:X, or the cDNA in a deposited cDNA clone may be analyzed using the default parameters of the DNASTAR computer algorithm (DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715 USA; http://www.dnastar.com/).

Polypeptide regions that may be routinely obtained using the DNASTAR computer algorithm include, but are not limited to, Garnier-Robson alpha-regions, beta-regions, turn-regions, and coil-regions, Chou-Fasman alpha-regions, beta-regions, and turn-regions,

Kyte-Doolittle hydrophilic regions and hydrophobic regions, Eisenberg alpha- and beta-amphipathic regions, Karplus-Schulz flexible regions, Emini surface-forming regions and Jameson-Wolf regions of high antigenic index. Among highly preferred polynucleotides of the invention in this regard are those that encode polypeptides comprising regions that combine several structural features, such as several (e.g., 1, 2, 3 or 4) of the features set out above.

Additionally, Kyte-Doolittle hydrophilic regions and hydrophobic regions, Emini surface-forming regions, and Jameson-Wolf regions of high antigenic index (i.e., containing four or more contiguous amino acids having an antigenic index of greater than or equal to 1.5, as identified using the default parameters of the Jameson-Wolf program) can routinely be used to determine polypeptide regions that exhibit a high degree of potential for antigenicity. Regions of high antigenicity are determined from data by DNASTAR analysis by choosing values which represent regions of the polypeptide which are likely to be exposed on the surface of the polypeptide in an environment in which antigen recognition may occur in the process of initiation of an immune response.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively consisting of, an amino acid sequence that displays a functional activity of the polypeptide sequence of which the amino acid sequence is a fragment.

By a polypeptide demonstrating a "functional activity" is meant, a polypeptide capable of displaying one or more known functional activities associated with a full-length (complete) protein of the invention. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for a polypeptide.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

In preferred embodiments, polypeptides of the invention comprise, or alternatively consist of, one, two, three, four, five or more of the antigenic fragments of the polypeptide of

SEQ ID NO:Y, or portions thereof. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Table 4.

Sequence/ Contig ID	Epitope
462108	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 461 as residues: Ile-1 to Arg-9, Val-26 to Val-41, Met-46 to Cys-51, Trp-88 to Gln-93, Glu-124 to Trp-130, Gly-339 to Pro-344.
503446	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 462 as residues: Leu-54 to Leu-60.
507841	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 463 as residues: Tyr-39 to Trp-44.
509287	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 464 as residues: Arg-6 to Val-12, Thr-38 to Asn-43, Arg-69 to Asp-74, Trp-87 to Lys-97, His-136 to Met-142, Ala-149 to Lys-160.
509672	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 465 as residues: Ser-33 to Cys-39.
524112	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 469 as residues: Asp-1 to Gly-6, Pro-30 to Gly-40, Leu-46 to Asn-52, Asp-54 to Gly-61.
525971	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 470 as residues: Pro-13 to Arg-21, Leu-30 to Thr-35, Pro-43 to Ser-51.
527156	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 471 as residues: Ala-2 to Pro-7.
532502	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 472 as residues: Lys-1 to Ser-6.
533459	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 473 as residues: Gly-1 to Trp-7, Ile-155 to Gly-163.
533551	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 474 as residues: Lys-15 to Leu-20.
537850	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 475 as residues: Ile-43 to Leu-49, Cys-85 to Lys-92, Phe-138 to Leu-144.
537925	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 476 as residues: Gln-17 to Ser-24, Ala-47 to Asn-52.
540802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 479 as residues: Leu-3 to Trp-9, Arg-20 to Phe-29, Glu-58 to Gln-65.
540989	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 480 as residues: Ser-52 to Gly-57, Thr-64 to Asn-70.
540997	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 481 as residues: Ile-1 to Thr-11.
548735	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 482 as residues: Gln-17 to Asn-22, Ser-38 to Pro-45, Asn-75 to Leu-84, Glu-97 to Pro-110.
549709	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 483 as residues: Phe-65 to Trp-77.
550007	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 484 as residues: Ser-4 to Ser-13, Leu-22 to Cys-40, Gly-42 to Gly-50, Thr-88 to Glu-97, Leu-184 to Gln-190, Pro-206 to Gly-211.
550118	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 485 as residues: Gly-1 to Gly-7, Trp-10 to Met-24, Gln-91 to Gly-98.
550870	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 487 as residues: Arg-26 to Arg-33, Gln-47 to Asn-52, Trp-61 to Ser-71, Gly-93 to Trp-100.
553765	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 489 as residues: Thr-8 to Thr-19, Arg-108 to Ser-115, Ser-117 to Arg-128, Phe-143 to Tyr-155, Leu-171 to Arg-177, Asn-182 to Gly-187, Gly-195 to Ser-200, Arg-232 to Thr-

	248, Pro-287 to Arg-293.
554050	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 490 as residues: Asp-49 to Lys-54, Glu-80 to Glu-86, Lys-121 to Leu-126, Thr-160 to Val-165, Ile-176 to Gly-181.
554186	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 491 as residues: Gln-1 to Cys-6, Asn-17 to Ala-24, Ala-157 to Asp-162, Ser-180 to Asp-185, Leu-219 to Thr-227, Lys-239 to Ile-246, Pro-266 to Asp-271.
554716	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 492 as residues: Thr-2 to His-10, Ser-51 to Ser-58, Ile-84 to Lys-89.
556791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 493 as residues: Asp-31 to Lys-37, Ser-58 to Phe-63, Lys-70 to Thr-79, Asp-100 to Ile-108.
557121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 494 as residues: Leu-29 to Gly-35, Ser-39 to Ala-47, Gln-91 to Arg-107.
557199	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 495 as residues: Ser-2 to His-12, Ser-14 to Ser-24, Gly-47 to Tyr-52, Pro-115 to Gly-126.
557293	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 496 as residues: Pro-14 to Gly-21, Pro-25 to Gly-36, Ala-43 to Gly-48, Pro-53 to Gly-78, Arg-90 to Asp-96, Pro-98 to Gly-103, Gln-117 to His-123, Ala-154 to Tyr-161.
558423	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 499 as residues: Gln-43 to Ile-49, Ala-106 to His-113, Glu-151 to Lys-156, Ala-186 to Arg-191, Lys-212 to Leu-223.
558465	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 500 as residues: Arg-1 to Arg-7, Gln-14 to Glu-22, Lys-52 to Gln-57, Lys-89 to Gly-96, Gly-103 to Ser-112, Ser-153 to His-168.
558778	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 502 as residues: His-2 to Ser-18.
558818	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 503 as residues: Asp-1 to His-9.
572571	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 505 as residues: Ser-1 to Pro-6, His-26 to Gly-31, Pro-36 to Lys-42, Pro-65 to Val-71.
575525	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 506 as residues: Arg-10 to Pro-19, Thr-34 to Gly-44.
580659	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 507 as residues: Val-17 to Ile-24.
583650	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 508 as residues: Ser-10 to Pro-19, Pro-26 to Ala-31.
585791	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 510 as residues: Ser-40 to Tyr-50, Pro-95 to Thr-125, Lys-131 to Ile-142, Thr-165 to Arg-178.
587229	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 511 as residues: Glu-51 to Gly-56, Cys-75 to Lys-87, Pro-98 to Cys-107, Ser-115 to Glu-120, Ala-139 to Gln-155.
587246	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 512 as residues: Glu-1 to Val-9, Pro-66 to Thr-73, Phe-84 to Trp-93.
592154	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 515 as residues: Pro-17 to Tyr-28, Arg-62 to Cys-68, Lys-75 to Thr-87.
598665	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 517 as residues: Leu-102 to Gln-108, Ser-114 to Asn-123, Asn-155 to Arg-160, Thr-169 to Pro-175, Ile-201 to Gln-207, Ser-236 to Ala-249, Asp-257 to Trp-262, Pro-275 to Gly-282, Pro-320 to Gln-336, Leu-386 to Arg-391.
604719	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 518 as residues: Pro-14 to Cys-25, Val-104 to Ile-110, His-116 to Gln-122, Ser-130 to Glu-142, Asn-162 to Asn-168, Arg-185 to Ile-191, Ser-210 to Lys-217.

612689	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 519 as residues: Lys-22 to Thr-29, Asp-39 to Ala-44, Arg-60 to Ser-65.
612980	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 520 as residues: Leu-37 to Gly-44.
615134	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 521 as residues: His-23 to Gly-33, Cys-89 to Arg-95, Asn-127 to Ala-136, Arg-177 to Gln-183.
616064	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 522 as residues: Trp-7 to Ser-14, Cys-69 to Glu-80.
616096	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 523 as residues: Pro-11 to Arg-34.
616926	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 524 as residues: Arg-25 to His-39.
634923	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 525 as residues: Tyr-20 to Ser-26, Ser-48 to Asn-54.
647531	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 527 as residues: Asp-24 to Phe-30.
647699	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 529 as residues: Glu-85 to Glu-93, Pro-107 to Asn-116, Gln-185 to His-192.
651706	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 530 as residues: Ser-41 to Gly-47, Gln-63 to Val-71, Tyr-83 to Pro-90, Leu-123 to Ser-128, Pro-185 to Arg-190, Asp-203 to Asn-210, Lys-232 to Trp-237, Glu-243 to Ser-249, Gly-281 to Asn-289, Thr-306 to Gly-311.
654015	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 533 as residues: Phe-14 to Tyr-19.
657859	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 536 as residues: His-1 to Trp-10, Pro-12 to Ser-24.
662212	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 538 as residues: Pro-20 to Thr-47, Ser-54 to Pro-61.
662496	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 540 as residues: Thr-51 to Gly-63, Arg-65 to Phe-72, Phe-78 to Asp-86, Ser-89 to Gly-104.
670453	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 542 as residues: His-9 to Gln-14, Ile-112 to Gly-118, Arg-150 to Leu-157, His-187 to Gly-197, Pro-229 to Trp-235.
675028	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 543 as residues: Arg-1 to His-9, Asn-35 to Arg-40.
681325	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 544 as residues: Pro-15 to Arg-23.
683103	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 545 as residues: Arg-1 to Ser-7, Ser-37 to Gln-43, Pro-107 to Thr-119, His-146 to Asn-151, Gly-158 to Gln-177, Glu-201 to Lys-206, Thr-236 to Leu-242, Gly-265 to Arg-271.
684432	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 546 as residues: Asp-1 to Asn-7, Thr-72 to Gly-79, Val-94 to Gly-99, Arg-182 to Ala-191, Asn-203 to Ser-212.
688018	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 547 as residues: Glu-1 to Trp-11.
691522	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 549 as residues: Tyr-38 to Gly-45, Lys-102 to Leu-109, Lys-114 to Ser-119, Asp-161 to Gln-166, Gln-179 to Gly-188.
693706	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 550 as residues: Leu-57 to Phe-62, Leu-100 to Ser-105, Ile-119 to Pro-134, Asn-154 to Asn-165, Asp-173 to Lys-186, Leu-213 to Gly-222, Lys-225 to Glu-231, Asp-243 to Glu-248, Gln-307 to Lys-315, Glu-317 to Tyr-323, His-327 to Lys-334, Pro-362 to Arg-

	367, Lys-402 to Thr-409, Lys-446 to Glu-457, Arg-577 to Asn-587, Ser-619 to Arg-624, Ser-640 to Gly-646, Glu-654 to Gly-660, Pro-669 to Glu-674, Asn-694 to Lys-701, Ala-712 to Glu-725, His-749 to Asp-757.
694523	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 551 as residues: Thr-2 to Arg-9, Arg-17 to Glu-33.
697517	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 552 as residues: Val-21 to Leu-27, Glu-30 to His-36.
699054	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 553 as residues: Gln-1 to Gln-17, Leu-24 to Gly-36.
703402	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 555 as residues: Arg-47 to Arg-57, Gln-59 to Tyr-65, Pro-67 to Phe-75, Arg-92 to Phe-97, Glu-108 to Val-120.
703651	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 556 as residues: Lys-41 to His-51, Asp-65 to Lys-73.
704905	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 557 as residues: Pro-19 to Thr-27, Ala-63 to Ser-71, Leu-92 to Ala-97.
708515	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 559 as residues: Lys-25 to Gly-35, Pro-37 to Met-42, Glu-110 to Glu-119, Leu-123 to Gly-128.
710572	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 560 as residues: Trp-1 to Glu-8, Glu-14 to Met-24, Ala-38 to Val-50, Gly-72 to Leu-79.
710618	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 561 as residues: Lys-61 to Asp-66.
711810	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 562 as residues: Arg-1 to Ile-8, Pro-50 to Thr-62.
714933	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 563 as residues: Asp-59 to Ser-71, Asp-86 to Leu-99, Arg-118 to Tyr-123.
716331	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 564 as residues: Met-3 to Ser-9, Leu-86 to Ser-91.
717686	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 565 as residues: Arg-18 to Asn-25.
718187	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 566 as residues: Phe-24 to Lys-29.
719934	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 567 as residues: Ser-36 to Trp-41, Ser-55 to Asn-60, Thr-67 to Phe-74, Ser-87 to Thr-95, Lys-132 to Gln-144, Ala-186 to Gly-192, Pro-260 to Asn-265, Leu-289 to Tyr-295, Ala-336 to Gly-347, Gly-386 to Gln-393, Thr-400 to Ser-413.
722980	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 568 as residues: Arg-1 to Gly-9, Ala-54 to Asp-59.
723596	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 569 as residues: Glu-65 to Tyr-70.
724352	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 570 as residues: Val-6 to Asn-20, His-45 to Pro-56.
724904	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 573 as residues: Glu-4 to Leu-14, Arg-52 to Lys-58, Asp-60 to Ile-70, Val-85 to Asp-92, Pro-99 to Arg-111.
725642	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 574 as residues: Arg-1 to Thr-14, Pro-28 to Asp-33, Lys-92 to Leu-101.
726192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 575 as residues: Val-7 to Ser-15.
730930	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 577 as residues: Phe-12 to Thr-18, Leu-30 to Leu-36, Thr-56 to Ser-62, Ile-115 to Phe-120.
732386	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 579 as

	residues: Thr-1 to Leu-12, Gly-39 to Gln-44. Thr-52 to Pro-59. Ser-88 to Pro-95, Val-122 to Gln-132. Asp-139 to Glu-144. Ser-177 to Ala-182. Gln-200 to Gly-207.
732909	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 580 as residues: Glu-45 to Arg-51. Pro-107 to Lys-115.
733088	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 581 as residues: Phe-6 to Pro-13. Glu-24 to Asn-32. Arg-58 to Asn-64. Arg-87 to Ile-95.
734760	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 584 as residues: Glu-1 to Trp-13. Gln-15 to Asp-22.
735711	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 585 as residues: Gln-11 to His-19. Val-30 to Ile-36. Pro-63 to Ser-69. Gly-78 to Ser-83, Ser-92 to Tyr-97. Gln-155 to Glu-161. Gly-237 to Thr-244.
742413	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 586 as residues: Gly-47 to Tyr-52. Thr-56 to Leu-62. Ser-65 to Thr-76, Leu-103 to Asp-144. Lys-149 to Leu-154. Asn-190 to Ser-198.
742676	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 587 as residues: Asn-2 to Ala-7.
742781	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 588 as residues: Thr-40 to Val-45. Lys-59 to Ser-64.
743356	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 589 as residues: Gly-4 to Lys-10.
750986	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 592 as residues: Arg-1 to Lys-7. Asn-20 to Gln-27. Phe-49 to Asn-58. Glu-63 to Gln-69, Gln-73 to Thr-78. Gln-136 to Leu-141. Ala-145 to Lys-153.
751068	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 593 as residues: Thr-5 to Ser-11.
751164	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 594 as residues: Gly-24 to Gly-32.
751890	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 595 as residues: Ala-24 to Ser-29.
751991	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 596 as residues: Tyr-1 to Gly-21. Ala-23 to Thr-29.
752449	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 597 as residues: Ser-17 to Thr-25.
752504	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 598 as residues: Arg-11 to Pro-26. Ala-37 to Asp-45. Asp-51 to Val-59. Glu-80 to Asp-98. Pro-104 to Trp-112. Asp-114 to Phe-124. Pro-140 to Pro-147. Pro-153 to Ala-158.
752688	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 599 as residues: Gly-1 to Pro-9. Arg-26 to Asp-31, Asp-33 to Val-58, Pro-71 to Ala-77, Ser-87 to Gly-95.
752889	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 600 as residues: Thr-1 to Lys-10.
753150	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 601 as residues: His-16 to Glu-35. Leu-43 to Tyr-55. His-68 to Gly-75. Ser-83 to Leu-89. Glu-106 to Ser-248. Ser-250 to Glu-306.
754479	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 603 as residues: Leu-47 to Ala-52. Ser-60 to Arg-80.
757127	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 606 as residues: Thr-25 to Ser-36.
757495	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 608 as residues: Arg-1 to Asp-6. Gln-46 to Val-59, Arg-93 to Ser-101. Gln-103 to Val-111, Pro-114 to Ser-119. Arg-138 to Glu-144. Ala-206 to Thr-212. Asn-228 to Asn-236, Asp-245 to Val-253. Pro-264 to Asp-270. His-295 to Asp-302. Leu-339 to Glu-349.
757715	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 609 as

	residues: Pro-1 to Val-15, Phe-21 to Val-27.
760388	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 610 as residues: Thr-24 to Gln-29. Val-56 to Gly-61.
760433	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 611 as residues: Thr-17 to Gln-33. Pro-35 to Arg-46, Ser-51 to Ala-58. Ser-98 to Leu-104. Phe-126 to Gly-137. Arg-139 to Leu-144. Ser-147 to Glu-153. Ala-164 to Gly-172.
760545	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 612 as residues: Met-1 to Phe-6.
761566	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 613 as residues: Glu-38 to Gly-43.
761740	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 614 as residues: Pro-35 to Asn-42. Lys-79 to Lys-84. Phe-131 to Cys-136.
766686	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 617 as residues: His-36 to Arg-48.
767396	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 618 as residues: Gln-33 to Asp-44. Pro-58 to Thr-79.
767501	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 619 as residues: Asp-1 to His-6. His-27 to Lys-37. Asn-141 to His-147. Asp-233 to Thr-239.
767945	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 620 as residues: Leu-5 to Leu-15.
771415	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 622 as residues: Gly-1 to Gly-9.
772657	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 623 as residues: Arg-1 to Gly-7, Gly-9 to Pro-21, Gly-39 to Arg-49, Thr-68 to Asn-73, Asp-78 to Arg-85, Thr-107 to Gln-116, Gln-147 to Arg-163. Gln-172 to Lys-187. Gln-240 to His-270. Tyr-282 to Ser-290.
773193	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 625 as residues: Gly-1 to Glu-13. Thr-29 to Ser-41, Gln-112 to His-123. Arg-133 to Gly-143.
773710	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 626 as residues: Ala-89 to Gly-94, Gly-108 to Thr-116, Leu-162 to Ala-167, Pro-169 to Ser-176. Val-217 to Arg-222.
774283	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 627 as residues: Asp-47 to Thr-71. Asp-78 to Ser-86, Pro-98 to Cys-103. Val-120 to Thr-129.
774369	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 628 as residues: Tyr-20 to Gly-26. Thr-36 to Ser-41, Lys-58 to Thr-64.
774754	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 629 as residues: Cys-5 to Glu-27. Glu-51 to Leu-75, Leu-86 to Phe-93, Val-169 to Lys-182. Ile-200 to Gln-206. Ala-250 to Met-257, Ser-301 to Asn-313. Asp-333 to Glu-342, Leu-344 to Asp-359. Asp-370 to Glu-381, Ser-390 to Gln-396.
774823	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 630 as residues: Leu-6 to Gln-12.
775510	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 631 as residues: Ser-15 to Ala-22.
775640	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 633 as residues: Ser-18 to Tyr-28.
775802	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 634 as residues: Val-1 to Glu-7.
777470	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 635 as residues: Arg-1 to Thr-11. Ala-45 to Glu-52. Cys-76 to Thr-88. Ala-94 to Arg-105. Asp-170 to Phe-178.

779273	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 638 as residues: Glu-46 to Phe-51. Pro-88 to Phe-95. Gly-104 to Val-110.
779297	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 639 as residues: Leu-25 to Arg-36. Ala-55 to Ser-60. Arg-67 to Tyr-84. Met-94 to Ala-100.
779664	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 640 as residues: Arg-34 to Ile-44. Ile-87 to Lys-108. Ile-128 to Met-139. Asp-143 to Gly-148.
781579	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 644 as residues: Gly-16 to Ser-37. Phe-83 to Asp-90.
782052	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 645 as residues: Arg-1 to Cys-12. Glu-15 to Pro-24.
782393	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 646 as residues: Tyr-1 to Gly-13. Gly-32 to Ser-39. Glu-71 to Ser-77.
782907	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 647 as residues: Ala-3 to Asp-22.
783220	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 648 as residues: Ser-8 to Leu-28. Asp-30 to Glu-43. Arg-48 to Pro-70. Glu-87 to Arg-97. Lys-106 to Pro-114.
783300	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 649 as residues: Thr-1 to Trp-15.
783938	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 650 as residues: Leu-13 to Arg-18. Lys-62 to Val-70. Phe-98 to Arg-107.
784024	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 651 as residues: Val-2 to Glu-7. Cys-15 to Tyr-32. Pro-52 to Arg-59.
784575	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 652 as residues: Asn-39 to His-44. Asp-59 to Met-64.
785006	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 653 as residues: Tyr-1 to Thr-10. Pro-12 to Pro-21.
785237	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 655 as residues: Glu-22 to Gln-29.
786111	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 656 as residues: Ala-1 to Thr-20. Cys-50 to Cys-63. Arg-70 to His-76. Pro-85 to Trp-93.
787036	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 657 as residues: Thr-31 to Gln-44. Ser-52 to Glu-57. Phe-73 to Ala-80. Thr-87 to Ser-94.
789626	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 660 as residues: Val-68 to Ser-74.
789703	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 661 as residues: Thr-8 to Lys-28. Cys-88 to Lys-96, Arg-98 to Ile-106, Asn-139 to Val-146, Glu-149 to Glu-162. Ser-172 to Arg-179. His-191 to Arg-196, Glu-214 to Leu-219, Glu-225 to Lys-260.
790848	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 663 as residues: Ser-47 to Lys-54.
790912	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 665 as residues: Gly-1 to Met-8. Arg-36 to Arg-43.
791386	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 666 as residues: Ser-20 to Gly-31, Phe-35 to Trp-45. Glu-52 to Trp-65, Thr-70 to Asp-78, Ala-86 to Gly-99. Glu-101 to Ala-106. Pro-112 to Trp-122.
791598	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 667 as residues: Arg-19 to Ala-30.
791619	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 668 as residues: Pro-39 to Asn-48. Ser-58 to Ile-69. Pro-72 to Gln-80. Ser-82 to Lys-103, Glu-111 to Pro-122. Ser-128 to Gln-157. Glu-172 to Ser-177.
791628	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 669 as

	residues: Ala-33 to Asp-39. Ala-81 to Ser-100.
791751	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 670 as residues: Arg-63 to Arg-72.
792557	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 671 as residues: Lys-51 to Arg-58.
792568	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 672 as residues: Glu-1 to Cys-9. Thr-65 to Leu-70. Asp-86 to Arg-92. Pro-132 to His-138.
793507	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 676 as residues: Pro-20 to Thr-25. Arg-60 to Asp-65.
793546	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 677 as residues: Pro-51 to Ser-56, Ser-62 to Thr-71, Leu-100 to Tyr-105, Pro-179 to Ala-186, Pro-200 to Lys-205, Glu-238 to Glu-243, Lys-250 to Tyr-261, Gln-317 to Gln-322.
793559	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 678 as residues: Gly-43 to Gln-48.
794121	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 680 as residues: Ala-1 to Glu-9. Gly-21 to Lys-29. Leu-31 to Lys-46. Pro-79 to Pro-85. Ser-111 to Leu-121. Arg-123 to Asn-138. Pro-146 to Arg-156.
794295	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 681 as residues: Arg-14 to Asp-21. Glu-29 to Ala-35. Thr-61 to Lys-66, Arg-91 to Gly-102, Ser-131 to Arg-144.
795241	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 682 as residues: Pro-5 to Asp-14. Pro-66 to Asn-74, Pro-83 to Asp-89, Glu-99 to His-104, Glu-116 to Ala-124. Leu-135 to Ala-142.
795286	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 683 as residues: Asn-13 to Thr-20.
795637	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 684 as residues: Phe-5 to Gly-17, His-68 to Glu-74, Pro-198 to Leu-203, Glu-205 to Lys-211. Val-245 to Trp-256. Phe-292 to Asn-297. Asp-325 to Gly-330. Gly-344 to Gln-360. Gly-379 to Gly-385. Gly-418 to Ser-427.
796301	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 685 as residues: Ala-4 to Asp-11. Ala-34 to Ser-43, Asp-50 to Ser-64. Arg-78 to Thr-95, Pro-104 to Ser-110. Ser-140 to Arg-148.
796590	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 688 as residues: Met-34 to Asp-42. Tyr-51 to Ala-56. Pro-67 to Leu-73. Ile-81 to Gly-88, Arg-166 to Val-172.
799783	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 689 as residues: Val-1 to Arg-9. Arg-26 to Gln-32. Arg-51 to Leu-63.
799784	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 690 as residues: Lys-19 to Arg-25. Phe-44 to Gln-49. Leu-70 to Ser-76.
799786	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 692 as residues: Thr-1 to Arg-19. Pro-22 to Arg-39. Pro-51 to Cys-78.
799800	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 694 as residues: Arg-8 to Ser-15. Thr-22 to Gly-43.
799808	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 695 as residues: Tyr-21 to Ser-26.
799977	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 696 as residues: Ser-28 to Ser-42.
800189	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 698 as residues: Arg-3 to Gln-14. Gln-18 to Gln-25. Lys-30 to Ser-36. Lys-75 to Thr-86. Glu-100 to Ser-107.
800589	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 699 as residues: Asp-1 to Asn-9. Lys-18 to Trp-31.

800811	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 700 as residues: Ser-1 to Leu-36, Leu-45 to Pro-78, Pro-80 to Thr-88, Leu-98 to Gly-123, Pro-126 to Ser-133, Asn-136 to Ser-149, Pro-160 to Gly-191.
805818	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 703 as residues: His-20 to Pro-25, Arg-72 to Ala-85, Pro-87 to His-102, Pro-128 to Arg-137, Met-145 to Leu-152, Arg-193 to Gly-199, Gly-269 to Arg-276, Pro-279 to Glu-284.
806579	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 705 as residues: Pro-54 to Ser-61, Leu-68 to Gln-74.
812314	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 709 as residues: Arg-1 to Gly-7, Leu-9 to Ser-16, Arg-25 to Cys-35.
812443	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 710 as residues: Lys-10 to Lys-24, Gln-30 to Glu-38, Thr-51 to Glu-62, Lys-85 to Tyr-90, Glu-171 to Trp-176, Gly-182 to Pro-188.
812498	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 711 as residues: Gly-57 to Ser-67.
813079	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 713 as residues: Asn-1 to Tyr-6, Met-24 to Asp-31, Glu-129 to Gly-135, Asp-164 to Lys-169.
815889	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 714 as residues: Lys-47 to Ile-64, Asp-72 to Glu-77, Lys-105 to Ala-111, Asp-145 to Gly-150, Asn-167 to Glu-172, Phe-180 to Gln-190.
824358	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 715 as residues: Ser-27 to Lys-32, Tyr-53 to Val-58, Lys-84 to Cys-89, Tyr-98 to Val-103, Asn-142 to Ser-156, Lys-162 to Glu-171, Ala-191 to Glu-231, Ala-237 to Tyr-247, Arg-254 to Thr-260, Tyr-267 to Ser-282.
826144	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 716 as residues: Ser-2 to Gly-7, Tyr-18 to Phe-26, Lys-39 to Gly-57, Gly-100 to Pro-106, Asn-109 to Ser-116, Tyr-119 to Ile-125, Pro-151 to Phe-157.
826558	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 717 as residues: Lys-4 to Ile-13, Arg-57 to His-62, Arg-68 to Gly-74.
827471	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 718 as residues: Lys-59 to Phe-69, Gln-98 to Thr-108, Pro-175 to Val-185, Asn-195 to Asp-206, Glu-214 to Gly-222, Ser-233 to Arg-240, Thr-258 to Thr-263, Pro-267 to Glu-272, Pro-278 to Glu-283, Pro-289 to Gly-294, Pro-300 to Gly-305, Pro-311 to Glu-316, Pro-322 to Gly-327, Pro-333 to Glu-338, Pro-344 to Ala-351.
827716	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 719 as residues: Lys-30 to Thr-37, Tyr-42 to Gly-54, Arg-93 to Thr-107, Pro-109 to Arg-116.
827722	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 720 as residues: Lys-1 to Lys-18.
827727	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 721 as residues: Lys-6 to Lys-24, Gln-50 to Glu-55, Arg-75 to Arg-90.
828238	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 722 as residues: Ser-78 to Trp-84, Pro-87 to Leu-94.
828573	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 723 as residues: Leu-9 to Thr-18, Leu-32 to Lys-37, Ser-45 to Leu-51, Val-80 to Glu-97, Pro-101 to Asp-108, Ala-115 to Gly-124, Ser-133 to Tyr-144, Glu-158 to Ser-165.
828848	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 726 as residues: Leu-8 to Ser-15, Arg-50 to Val-55, Gln-82 to Asp-88, Leu-96 to Ile-103, Thr-136 to Trp-141.
828929	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 727 as residues: His-2 to Leu-11, Glu-27 to Met-34, Ala-57 to Ser-72, Asn-119 to Phe-126.

829192	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 730 as residues: Ala-16 to Trp-28, Pro-36 to Gln-42, Glu-45 to Trp-50, Arg-137 to Ser-142, Ser-148 to Leu-153, Ile-178 to Gly-183, Asp-235 to Tyr-243.
829310	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 731 as residues: Cys-4 to Cys-14, Gly-86 to Ser-97.
829319	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 732 as residues: Asp-49 to Glu-54.
829459	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 733 as residues: His-1 to Thr-9.
829527	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 734 as residues: Gly-1 to Arg-8.
829736	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 735 as residues: Ala-1 to Lys-11, Arg-21 to Ser-26, Ser-45 to Ser-55, Tyr-115 to Asp-120, Asp-131 to Ile-145, Gln-147 to Asp-152, Ser-224 to Ser-231, Lys-252 to Glu-263, Ser-323 to Ser-332, His-341 to Asn-347.
830552	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 736 as residues: Phe-65 to Trp-73, Arg-87 to Gly-92, Gly-107 to Lys-112, Pro-177 to Thr-186.
830566	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 737 as residues: Pro-8 to Lys-19.
830569	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 739 as residues: Ser-37 to Trp-42, Ser-56 to Asp-61, Thr-68 to Asn-74, Lys-107 to Pro-113, Trp-133 to Arg-138, Asp-211 to Val-216, Pro-255 to Glu-260, Ser-293 to Ser-298, Cys-312 to Lys-322, Ser-374 to Asn-380, Gly-389 to Ile-399, Ser-403 to Ser-409, Ser-451 to Ser-462.
830583	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 740 as residues: Ala-5 to Gly-21, Gln-28 to Arg-37, Arg-67 to Ala-76, Glu-93 to Ala-100, Glu-117 to Arg-124, Lys-131 to Gly-145, Arg-152 to Met-160, Asp-176 to Glu-182, Asp-194 to Glu-203, Asp-231 to Glu-243, Lys-250 to Arg-257.
830716	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 744 as residues: Ala-5 to Arg-12, His-36 to Tyr-42, His-60 to Cys-75, Arg-87 to Gly-104, His-122 to Ser-140, Ser-163 to Pro-168, Thr-176 to Pro-181, Arg-195 to Pro-201.
830792	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 745 as residues: Cys-36 to Trp-43, Asn-113 to Ser-123, Pro-148 to Val-154, Glu-167 to Ser-172.
830893	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 746 as residues: Pro-33 to Trp-38, Arg-40 to Glu-46, Val-53 to Glu-58, Leu-66 to Leu-81, Leu-93 to Gln-98, Ile-145 to Asp-152.
831043	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 748 as residues: Glu-5 to Tyr-12, Ser-27 to Tyr-35.
831173	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 751 as residues: Ser-9 to Ser-14, Leu-41 to Gly-53, Thr-64 to Asn-71, Glu-78 to Thr-84.
831255	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 752 as residues: Gln-10 to Gly-21, Pro-39 to Pro-45.
831327	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 753 as residues: Gln-29 to Ile-42, Pro-45 to Ser-53, Cys-72 to Ser-77, Glu-98 to Ser-104, Asp-112 to Ser-122, Lys-130 to Ser-136, Ser-152 to Cys-162.
831493	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 754 as residues: Cys-1 to Gly-6, Pro-8 to Gln-19, Ser-29 to Cys-36, Pro-43 to Glu-64, Glu-70 to Thr-85.
831500	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 755 as residues: Ser-13 to Ala-25, Ser-64 to Gly-78, Glu-81 to Gln-89.
831502	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 757 as

	residues: Pro-19 to Phe-26. Pro-29 to Gly-34. Pro-50 to Ser-55.
831508	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 758 as residues: Asp-7 to Ser-14. Ser-42 to Ser-57.
831509	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 759 as residues: Gly-7 to Leu-13.
831520	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 760 as residues: Ser-17 to Gly-25.
831547	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 761 as residues: Ser-4 to Arg-10. Thr-89 to Trp-98. Thr-118 to Cys-124.
831847	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 764 as residues: Leu-27 to Lys-43.
831893	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 765 as residues: Lys-1 to Ser-18. Ile-20 to Val-27. Asp-44 to Thr-60.
831923	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 768 as residues: Pro-25 to Ser-33. Gln-113 to Ser-122. Trp-147 to Tyr-158. Ser-187 to Ala-198. His-201 to Gly-209. Pro-223 to Gly-228. Glu-233 to Gly-238.
831959	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 769 as residues: Tyr-46 to Gly-51.
832008	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 770 as residues: Ala-29 to Pro-48. Phe-79 to Thr-87. Glu-94 to Cys-101. Glu-111 to Asp-116.
832110	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 772 as residues: Val-2 to Leu-13. Ile-17 to Asn-22. Pro-49 to Ser-54, Ser-58 to Asp-74. Phe-107 to Ser-113. Gln-149 to Ser-159. Pro-166 to Lys-183. Ser-223 to Lys-229. Arg-251 to Glu-267. Ala-269 to Arg-275.
832146	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 773 as residues: Lys-26 to Ala-37. Pro-46 to Asn-52. Glu-137 to Pro-147. Ser-171 to Ser-185.
832189	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 774 as residues: Arg-20 to Asp-30. Pro-48 to Gly-53. Pro-67 to Gly-74.
832393	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 778 as residues: Gly-22 to Cys-29. Leu-52 to Phe-57. Phe-67 to Thr-73.
832448	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 781 as residues: Gly-2 to Arg-9. Leu-20 to Arg-28. Asp-33 to Arg-43. Lys-127 to Glu-132. His-146 to Pro-183.
832532	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 782 as residues: Val-4 to Ser-9. Lys-74 to Leu-79. Pro-95 to Lys-100. Asn-112 to Ile-117. Glu-129 to Ala-140. Asp-152 to Leu-158.
832621	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 783 as residues: Asp-17 to Glu-24. Glu-37 to Asn-44. Ile-53 to Gln-63. Glu-74 to Asp-82. Gln-91 to Lys-97. Leu-99 to Ile-104. Thr-114 to Ser-120.
832622	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 784 as residues: Leu-17 to Lys-36.
835327	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 785 as residues: Thr-40 to Gly-47.
835695	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 786 as residues: Gly-1 to Ile-11. Thr-23 to Ser-29.
835857	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 787 as residues: Leu-42 to Ser-54. Asp-82 to Ala-91. Lys-103 to Leu-111. Lys-117 to Asn-123. Glu-160 to Gln-165. Glu-183 to Val-192. Leu-225 to Lys-231. Lys-247 to Thr-255. Lys-279 to Asn-293. Leu-295 to Asn-303. Val-305 to Asn-317. Ile-360 to Cys-370. Leu-373 to Ala-385. Gln-413 to Ala-435. Pro-465 to Thr-489. Pro-491 to Gly-502. Pro-526 to Glu-534. Gln-550 to Val-559.

836183	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 788 as residues: Arg-57 to Thr-62.
836190	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 789 as residues: Val-34 to Ser-40.
836196	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 790 as residues: Arg-51 to Leu-57. Leu-61 to Ser-70. Ser-77 to Ser-84.
836253	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 791 as residues: Ser-1 to Thr-11.
836372	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 792 as residues: Gly-13 to Ser-30. Thr-38 to Trp-44. Ser-60 to Tyr-66. Asp-92 to Gln-99.
837445	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 794 as residues: Asn-1 to Gln-9, Lys-22 to Met-28. Gln-66 to Ser-73, Gln-76 to Gly-87, Ser-92 to Asp-99.
837620	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 795 as residues: Gln-11 to Gly-18. Ser-39 to Gln-44.
837995	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 797 as residues: Ser-44 to Ser-53. Thr-66 to Ser-71.
838237	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 799 as residues: Glu-62 to Asp-67. Gly-79 to Gly-85.
838700	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 800 as residues: Ser-88 to Lys-109. Lys-132 to Ile-137. Thr-158 to Asn-165. Asp-175 to Arg-191. Leu-199 to Gln-206. Leu-217 to Asp-222. Ser-229 to Ile-235. Gln-266 to Asn-271. Thr-293 to Gly-301. Tyr-321 to Asn-327. Phe-340 to Gln-348. Glu-415 to Asp-422. Gly-432 to Ser-439. Pro-443 to Arg-455. Asn-463 to Ser-470. Ser-478 to Cys-497. Ala-505 to Glu-552. Lys-558 to Lys-581.
839096	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 802 as residues: Arg-1 to Ser-17.
839588	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 804 as residues: Arg-41 to Glu-48.
839589	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 805 as residues: Arg-6 to His-13. Pro-69 to Glu-76.
839733	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 806 as residues: His-25 to His-31. Ser-61 to Gly-67. Pro-73 to Ala-80, Glu-123 to Ser-128. Glu-141 to Arg-149. Leu-162 to Gly-176. Ser-197 to Gly-204, Arg-222 to Asn-232. Gln-234 to Trp-242. Thr-250 to Val-257. Val-261 to Ala-271. Asp-301 to Thr-312. Pro-346 to Leu-352. Pro-355 to Cys-371. Ala-382 to Gly-394. Leu-435 to Asp-441. Pro-455 to Leu-460.
839874	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 807 as residues: Arg-98 to Thr-104. Gln-117 to Lys-122. Tyr-250 to Leu-262. Glu-296 to Lys-301.
840017	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 808 as residues: Ile-1 to Asp-6, Ser-42 to Asp-54, Ser-157 to Asn-166. Gly-188 to Ile-193. Glu-203 to Asp-208. Thr-236 to Lys-249. His-272 to Gln-278, Asn-364 to Glu-373, Ser-383 to Arg-388. Pro-391 to Ile-399. Gln-404 to Gly-412. Lys-420 to His-431.
840124	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 809 as residues: Gln-1 to Gly-8. Pro-17 to Trp-22.
840617	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 811 as residues: Thr-1 to Arg-6, Leu-22 to Glu-30, Lys-47 to Phe-61, Pro-131 to Asp-136, Arg-156 to Thr-161, Gln-181 to Trp-189. Glu-225 to Asp-234. Pro-251 to Thr-258, Ala-273 to Ser-278. Thr-285 to Arg-320. Pro-372 to Tyr-378. Val-380 to Ser-386, Asp-453 to Asn-460.
840792	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 813 as residues: Ala-1 to Gly-7. Ile-17 to Gly-38. Asn-50 to Lys-58. Gln-61 to Gln-68. Ser-

	80 to Val-86. Asp-182 to Ser-190.
841325	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 816 as residues: Arg-28 to Glu-90. Phe-94 to Ser-104, Leu-123 to Lys-129. Lys-147 to Gly-152.
841713	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 817 as residues: Ser-36 to Arg-46. Thr-52 to Asp-64. Ser-69 to Gly-89. Ser-96 to Asp-102. Ile-106 to Phe-120. Val-136 to Thr-142. Gly-146 to Asp-169. Lys-176 to Phe-182. Asp-200 to Ser-206.
842454	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 820 as residues: Gly-41 to Gly-53. Gly-65 to Arg-77.
842768	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 821 as residues: Thr-7 to Thr-13. Arg-49 to Gln-55.
842999	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 822 as residues: Leu-25 to Glu-32.
843830	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 823 as residues: Asp-24 to Asp-31. Gly-37 to Thr-47. Gly-55 to Ala-60. Gly-91 to Asn-107. Glu-113 to Glu-120.
844723	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 824 as residues: Gly-1 to Gly-7. Gly-14 to Gly-20.
844868	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 825 as residues: Pro-19 to Gly-40. Lys-54 to Ala-60. Lys-69 to Asn-74. Asn-80 to Pro-94.
845373	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 827 as residues: Tyr-3 to Gly-11. Arg-68 to Trp-76. Pro-82 to Ile-91. Asn-138 to Ala-144. Arg-169 to Lys-175. Ser-180 to Glu-192. Ile-421 to Ser-427.
845412	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 828 as residues: Cys-24 to Gly-35. Ala-42 to Glu-47. Gln-181 to Asp-188. Pro-277 to His-292.
HISED43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 829 as residues: Lys-1 to Trp-6. Gln-9 to Gln-16. Gly-66 to Val-71. Lys-74 to Trp-82.
HOSEQ76R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 830 as residues: Ser-36 to Gly-48.
HISDS43R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 831 as residues: Ser-28 to Arg-36.
HPJDY28R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 832 as residues: Cys-9 to His-14.
HISDW59R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 837 as residues: Ile-4 to Val-9.
HTPGD92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 839 as residues: Ser-9 to Pro-14.
HHFLB69R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 840 as residues: Pro-1 to Gly-6. Pro-20 to Arg-25. Ala-45 to Ser-50.
HPDEH50R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 841 as residues: Ser-24 to Ser-29.
HMTMA16 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 842 as residues: Cys-4 to Gly-11. Ile-59 to Gln-64. Asn-85 to Lys-90. Glu-94 to Lys-99.
HTPGL88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 844 as residues: Ala-30 to Gly-42. Leu-44 to Lys-50. Gln-60 to Asp-68. Gln-78 to Ser-84.
HMCIA86R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 845 as residues: Gly-39 to Ser-45. Arg-52 to Arg-58.
HDTFE89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 847 as residues: Glu-25 to Gln-32.
HTLHH34R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 850 as residues: Phe-11 to Ser-22. Ser-79 to Lys-86. His-97 to Asp-102.

HCCMA63R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 855 as residues: Gly-1 to Gly-13.
HE8EZ78R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 856 as residues: Ala-1 to Leu-7, Ile-14 to Gln-22, Glu-39 to Asp-44, Leu-76 to Val-84, Asn-89 to Leu-95, Pro-98 to Glu-103.
HALSD82R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 858 as residues: Asn-1 to Asp-6, Thr-19 to Cys-31, Glu-33 to Trp-39, Gly-56 to Asp-69, Met-84 to His-106, Lys-112 to His-118.
H2LAS44R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 859 as residues: His-10 to Gln-18, Ser-79 to Gly-89.
HTXPA42R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 860 as residues: Arg-1 to Lys-6, Asn-31 to Lys-39.
HAHEJ39R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 862 as residues: Asp-8 to Gly-14, Gly-19 to Ser-29, Arg-67 to Gly-72.
HOEMQ04R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 863 as residues: Lys-12 to Arg-21, Tyr-57 to Pro-71.
HOENU56R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 865 as residues: Leu-9 to Leu-15.
HAGGB37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 866 as residues: Asn-32 to His-38.
HAHDO57R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 868 as residues: Gly-1 to Gly-7, Gly-17 to Ser-28.
HTPCT95R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 871 as residues: Glu-33 to Trp-40, Tyr-48 to His-56.
HCCMD33R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 873 as residues: Glu-9 to Gly-14, Cys-33 to Lys-44.
HCE4L96R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 875 as residues: Gln-1 to Arg-8, Arg-13 to Ser-30, His-38 to Tyr-44.
HTPGL86R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 876 as residues: Gln-47 to Cys-53, Asn-66 to Cys-71.
HWDAK95 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 878 as residues: His-17 to Gln-26, Met-28 to His-39, Pro-48 to Gly-58.
HE9DG72R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 879 as residues: Val-29 to Lys-34, Thr-50 to Gly-56.
HDPOY89R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 880 as residues: Gln-1 to Met-11, Pro-26 to Ser-37, Pro-55 to His-60, Lys-83 to Thr-99.
HAHEJ13R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 881 as residues: Glu-12 to Ser-17.
HCFCM83R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 883 as residues: Glu-19 to Ala-26.
HBMBJ92R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 891 as residues: Leu-22 to Gly-27, Glu-33 to Val-38.
HCGBC37R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 892 as residues: Phe-26 to Val-31, Pro-35 to Arg-42.
HCROI22R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 893 as residues: Pro-5 to Ser-14, Ser-25 to Leu-30.
HDTLK21R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 894 as residues: Pro-11 to Asn-17.
HEGAD29R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 898 as residues: Glu-1 to His-6, Gly-19 to Trp-31.
HFKHC10R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 899 as residues: Val-12 to Asn-18, Lys-30 to Glu-38.
HNHGQ70R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 909 as

	residues: Pro-6 to Ala-16. Ala-61 to Met-68. Pro-72 to Ala-77. Ser-88 to His-93. Thr-113 to Ser-118.
HOSMV19R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 910 as residues: Pro-12 to Leu-18.
HULEB88R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 913 as residues: Glu-11 to Leu-17. Leu-36 to Thr-41.
HWLWG58 R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 917 as residues: Glu-1 to Cys-6.
HAIDL46R	Preferred epitopes include those comprising a sequence shown in SEQ ID NO. 918 as residues: His-1 to Asp-55. Asp-57 to His-74.

The present invention encompasses polypeptides comprising, or alternatively consisting of, an epitope of the polypeptide sequence shown in SEQ ID NO:Y, or an epitope of the polypeptide sequence encoded by the cDNA in the related cDNA clone contained in a deposited library or encoded by a polynucleotide that hybridizes to the complement of an epitope encoding sequence of SEQ ID NO:X, or an epitope encoding sequence contained in the deposited cDNA clone under stringent hybridization conditions, or alternatively, under lower stringency hybridization conditions, as defined supra. The present invention further encompasses polynucleotide sequences encoding an epitope of a polypeptide sequence of the invention (such as, for example, the sequence disclosed in SEQ ID NO:X), polynucleotide sequences of the complementary strand of a polynucleotide sequence encoding an epitope of the invention, and polynucleotide sequences which hybridize to this complementary strand under stringent hybridization conditions or alternatively, under lower stringency hybridization conditions, as defined supra.

The term "epitopes," as used herein, refers to portions of a polypeptide having antigenic or immunogenic activity in an animal, preferably a mammal, and most preferably in a human. In a preferred embodiment, the present invention encompasses a polypeptide comprising an epitope, as well as the polynucleotide encoding this polypeptide. An "immunogenic epitope," as used herein, is defined as a portion of a protein that elicits an antibody response in an animal, as determined by any method known in the art, for example, by the methods for generating antibodies described infra. (See, for example, Geysen et al., Proc. Natl. Acad. Sci. USA 81:3998- 4002 (1983)). The term "antigenic epitope," as used herein, is defined as a portion of a protein to which an antibody can immunospecifically bind its antigen as determined by any method well known in the art, for example, by the immunoassays described herein. Immunospecific binding excludes non-specific binding but does not necessarily exclude cross- reactivity with other antigens. Antigenic epitopes need not necessarily be immunogenic.

Fragments which function as epitopes may be produced by any conventional means. (See, e.g., Houghten. R. A., Proc. Natl. Acad. Sci. USA 82:5131-5135 (1985) further described in U.S. Patent No. 4,631,211.)

In the present invention. antigenic epitopes preferably contain a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8. at least 9, at least 10, at least 11, at least 12, at least 13, at least 14. at least 15, at least 20, at least 25, at least 30, at least 40, at least 50. and, most preferably, between about 15 to about 30 amino acids. Preferred polypeptides comprising immunogenic or antigenic epitopes are at least 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 amino acid residues in length. Additional non-exclusive preferred antigenic epitopes include the antigenic epitopes disclosed herein. as well as portions thereof. Antigenic epitopes are useful, for example, to raise antibodies, including monoclonal antibodies, that specifically bind the epitope. Preferred antigenic epitopes include the antigenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these antigenic epitopes. Antigenic epitopes can be used as the target molecules in immunoassays. (See, for instance, Wilson et al., Cell 37:767-778 (1984); Sutcliffe et al., Science 219:660-666 (1983)).

Similarly, immunogenic epitopes can be used, for example, to induce antibodies according to methods well known in the art. (See, for instance, Sutcliffe et al., supra; Wilson et al., supra; Chow et al., Proc. Natl. Acad. Sci. USA 82:910-914; and Bittle et al., J. Gen. Virol. 66:2347-2354 (1985). Preferred immunogenic epitopes include the immunogenic epitopes disclosed herein, as well as any combination of two, three, four, five or more of these immunogenic epitopes. The polypeptides comprising one or more immunogenic epitopes may be presented for eliciting an antibody response together with a carrier protein, such as an albumin, to an animal system (such as rabbit or mouse), or, if the polypeptide is of sufficient length (at least about 25 amino acids), the polypeptide may be presented without a carrier. However, immunogenic epitopes comprising as few as 8 to 10 amino acids have been shown to be sufficient to raise antibodies capable of binding to, at the very least, linear epitopes in a denatured polypeptide (e.g., in Western blotting).

Epitope-bearing polypeptides of the present invention may be used to induce antibodies according to methods well known in the art including, but not limited to, in vivo immunization, in vitro immunization, and phage display methods. See, e.g., Sutcliffe et al., supra; Wilson et al., supra, and Bittle et al., J. Gen. Virol., 66:2347-2354 (1985). If in vivo immunization is used, animals may be immunized with free peptide; however, anti-peptide antibody titer may be boosted by coupling the peptide to a macromolecular carrier, such as keyhole limpet hemacyanin (KLH) or tetanus toxoid. For instance, peptides containing cysteine residues may be coupled to a carrier using a linker such as maleimidobenzoyl- N-hydroxysuccinimide ester (MBS), while other peptides may be coupled to carriers using a more general linking agent such as glutaraldehyde. Animals such as rabbits, rats and mice are immunized with either free or carrier- coupled peptides, for instance, by intraperitoneal and/or intradermal injection of emulsions containing about 100 μg of peptide or carrier protein and Freund's adjuvant or any other adjuvant known for stimulating an immune response. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of anti-peptide antibody which can be detected, for example, by ELISA assay using free peptide adsorbed to a solid surface. The titer of anti-peptide antibodies in serum from an immunized animal may be increased by selection of anti-peptide antibodies, for instance, by adsorption to the peptide on a solid support and elution of the selected antibodies according to methods well known in the art.

As one of skill in the art will appreciate, and as discussed above, the polypeptides of the present invention , and immunogenic and/or antigenic epitope fragments thereof can be fused to other polypeptide sequences. For example, the polypeptides of the present invention may be fused with the constant domain of immunoglobulins (IgA, IgE, IgG, IgM), or portions thereof (CH1, CH2, CH3, or any combination thereof and portions thereof) resulting in chimeric polypeptides. Such fusion proteins may facilitate purification and may increase half-life in vivo. This has been shown for chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light

chains of mammalian immunoglobulins. See, e.g., EP 394,827; Traunecker et al., Nature, 331:84-86 (1988). Enhanced delivery of an antigen across the epithelial barrier to the immune system has been demonstrated for antigens (e.g., insulin) conjugated to an FcRn binding partner such as IgG or Fc fragments (see, e.g., PCT Publications WO 96/22024 and WO 99/04813). IgG Fusion proteins that have a disulfide-linked dimeric structure due to the IgG portion desulfide bonds have also been found to be more efficient in binding and neutralizing other molecules than monomeric polypeptides or fragments thereof alone. See, e.g., Fountoulakis et al., J. Biochem., 270:3958-3964 (1995).

Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or part thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP-A 0232 262.) Alternatively, deleting the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, D. Bennett et al., J. Molecular Recognition 8:52-58 (1995); K. Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).)

Moreover, the polypeptides of the present invention can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope

derived from the influenza hemagglutinin protein. (Wilson et al., Cell 37:767 (1984).)

Thus, any of these above fusions can be engineered using the polynucleotides or the polypeptides of the present invention.

Nucleic acids encoding the above epitopes can also be recombined with a gene of interest as an epitope tag (e.g., the hemagglutinin ("HA") tag or flag tag) to aid in detection and purification of the expressed polypeptide. For example, a system described by Janknecht et al. allows for the ready purification of non-denatured fusion proteins expressed in human cell lines (Janknecht et al., Proc. Natl. Acad. Sci. USA 88:8972- 897 (1991)). In this system, the gene of interest is subcloned into a vaccinia recombination plasmid such that the open reading frame of the gene is translationally fused to an amino-terminal tag consisting of six histidine residues. The tag serves as a matrix binding domain for the fusion protein. Extracts from cells infected with the recombinant vaccinia virus are loaded onto Ni2+ nitriloacetic acid-agarose column and histidine-tagged proteins can be selectively eluted with imidazole-containing buffers.

Additional fusion proteins of the invention may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling"). DNA shuffling may be employed to modulate the activities of polypeptides of the invention, such methods can be used to generate polypeptides with altered activity, as well as agonists and antagonists of the polypeptides. See, generally, U.S. Patent Nos. 5,605,793; 5,811,238; 5,830,721; 5,834,252; and 5,837,458, and Patten et al., Curr. Opinion Biotechnol. 8:724-33 (1997); Harayama, Trends Biotechnol. 16(2):76-82 (1998); Hansson, et al., J. Mol. Biol. 287:265-76 (1999); and Lorenzo and Blasco, Biotechniques 24(2):308- 13 (1998) (each of these patents and publications are hereby incorporated by reference in its entirety). In one embodiment, alteration of polynucleotides corresponding to SEQ ID NO:X and the polypeptides encoded by these polynucleotides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments by homologous or site-specific recombination to generate variation in the

polynucleotide sequence. In another embodiment, polynucleotides of the invention, or the encoded polypeptides, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of a polynucleotide encoding a polypeptide of the invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

As discussed herein, any polypeptide of the present invention can be used to generate fusion proteins. For example, the polypeptide of the present invention, when fused to a second protein, can be used as an antigenic tag. Antibodies raised against the polypeptide of the present invention can be used to indirectly detect the second protein by binding to the polypeptide. Moreover, because secreted proteins target cellular locations based on trafficking signals, polypeptides of the present invention which are shown to be secreted can be used as targeting molecules once fused to other proteins.

Examples of domains that can be fused to polypeptides of the present invention include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but may occur through linker sequences.

In certain preferred embodiments, proteins of the invention comprise fusion proteins wherein the polypeptides are N and/or C- terminal deletion mutants. In preferred embodiments, the application is directed to nucleic acid molecules at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to the nucleic acid sequences encoding polypeptides having the amino acid sequence of the specific N- and C-terminal deletions mutants. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fusion proteins may also be engineered to improve characteristics of the polypeptide of the present invention. For instance, a region of additional amino acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence during purification from the host cell

or subsequent handling and storage. Also, peptide moieties may be added to the polypeptide to facilitate purification. Such regions may be removed prior to final preparation of the polypeptide. The addition of peptide moieties to facilitate handling of polypeptides are familiar and routine techniques in the art.

Vectors. Host Cells. and Protein Production

The present invention also relates to vectors containing the polynucleotide of the present invention, host cells, and the production of polypeptides by recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

The polynucleotides of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the E. coli lac, trp, phoA and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region, a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in E. coli and other bacteria. Representative examples

of appropriate hosts include, but are not limited to. bacterial cells, such as E. coli, Streptomyces and Salmonella typhimurium cells: fungal cells, such as yeast cells (e.g., Saccharomyces cerevisiae or Pichia pastoris (ATCC Accession No. 201178)); insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN. Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection. or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

A polypeptide of this invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most

preferably, high performance liquid chromatography ("HPLC") is employed for purification.

Polypeptides of the present invention can also be recovered from: products purified from natural sources, including bodily fluids, tissues and cells, whether directly isolated or cultured: products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial. yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, polypeptides of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express polypeptides of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolization pathway is the oxidation of methanol to formaldehyde using O_2. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O_2. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. *See*, Ellis, S.B., *et al.*. *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J. *et al.*. *Yeast*

5:167-77 (1989); Tschopp, J.F., *et al.*. *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as. for example, a polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding a polypeptide of the invention, as set forth herein. in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., coding sequence), and/or to include genetic material (e.g., heterologous polynucleotide sequences) that is operably associated with polynucleotides of the invention, and

which activates, alters, and/or amplifies endogenous polynucleotides. For example, techniques known in the art may be used to operably associate heterologous control regions (e.g., promoter and/or enhancer) and endogenous polynucleotide sequences via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

In addition, polypeptides of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983. Proteins: Structures and Molecular Principles, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid, ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

Non-naturally occurring variants may be produced using art-known mutagenesis techniques, which include, but are not limited to oligonucleotide mediated mutagenesis, alanine scanning, PCR mutagenesis, site directed mutagenesis (*see, e.g.*, Carter *et al., Nucl. Acids Res. 13*:4331 (1986); and Zoller *et al., Nucl. Acids Res. 10*:6487 (1982)), cassette mutagenesis (*see, e.g.*, Wells *et al., Gene 34*:315

(1985)), restriction selection mutagenesis (*see, e.g.*, Wells *et al.*, *Philos. Trans. R. Soc. London SerA 317*:415 (1986)).

The invention additionally, encompasses polypeptides of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, $NaBH_4$; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The polypeptides may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Also provided by the invention are chemically modified derivatives of the polypeptides of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The polypeptides may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between

about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200; 500; 1000; 1500; 2000; 2500; 3000; 3500; 4000; 4500; 5000; 5500; 6000; 6500; 7000; 7500; 8000; 8500; 9000; 9500; 10,000; 10,500; 11,000; 11,500; 12,000; 12,500; 13,000; 13,500; 14,000; 14,500; 15,000; 15,500; 16,000; 16,500; 17,000; 17,500; 18,000; 18,500; 19,000; 19,500; 20,000; 25,000; 30,000; 35,000; 40,000; 50,000; 55,000; 60,000; 65,000; 70,000; 75,000; 80,000; 85,000; 90,000; 95,000; or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al., Appl. Biochem. Biotechnol. 56*:59-72 (1996); Vorobjev *et al., Nucleosides Nucleotides 18*:2745-2750 (1999); and Caliceti *et al., Bioconjug. Chem. 10*:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, e.g., EP 0 401 384, herein incorporated by reference (coupling PEG to G-CSF), see also Malik et al., Exp. Hematol. 20:1028-1035 (1992) (reporting pegylation of GM-CSF using tresyl chloride). For example, polyethylene glycol may be covalently bound through amino acid residues via a reactive group, such as, a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid

residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to a proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the protein either directly or by an intervening linker. Linkerless systems

for attaching polyethylene glycol to proteins are described in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys. 9*:249-304 (1992); Francis *et al., Intern. J. of Hematol. 68*:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ($ClSO_2CH_2CF_3$). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoreothane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol to proteins are described in WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

The number of polyethylene glycol moieties attached to each protein of the invention (*i.e.*, the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of

substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado *et al., Crit. Rev. Thera. Drug Carrier Sys.* 9:249-304 (1992).

The pancreatic cancer antigen polypeptides of the invention may be in monomers or multimers (i.e., dimers, trimers, tetramers and higher multimers). Accordingly, the present invention relates to monomers and multimers of the polypeptides of the invention, their preparation, and compositions (preferably, Therapeutics) containing them. In specific embodiments, the polypeptides of the invention are monomers, dimers, trimers or tetramers. In additional embodiments, the multimers of the invention are at least dimers, at least trimers, or at least tetramers.

Multimers encompassed by the invention may be homomers or heteromers. As used herein, the term homomer, refers to a multimer containing only polypeptides corresponding to the amino acid sequence of SEQ ID NO:Y or an amino acid sequence encoded by SEQ ID NO:X, and/or an amino acid sequence encoded by the cDNA in a related cDNA clone contained in a deposited library (including fragments, variants, splice variants, and fusion proteins, corresponding to any one of these as described herein). These homomers may contain polypeptides having identical or different amino acid sequences. In a specific embodiment, a homomer of the invention is a multimer containing only polypeptides having an identical amino acid sequence. In another specific embodiment, a homomer of the invention is a multimer containing polypeptides having different amino acid sequences. In specific embodiments, the multimer of the invention is a homodimer (e.g., containing polypeptides having identical or different amino acid sequences) or a homotrimer (e.g., containing polypeptides having identical and/or different amino acid sequences). In additional embodiments, the homomeric multimer of the invention is at least a homodimer, at least a homotrimer, or at least a homotetramer.

As used herein, the term heteromer refers to a multimer containing one or more heterologous polypeptides (i.e., polypeptides of different proteins) in addition to

the polypeptides of the invention. In a specific embodiment, the multimer of the invention is a heterodimer. a heterotrimer, or a heterotetramer. In additional embodiments, the heteromeric multimer of the invention is at least a heterodimer, at least a heterotrimer, or at least a heterotetramer.

Multimers of the invention may be the result of hydrophobic, hydrophilic, ionic and/or covalent associations and/or may be indirectly linked, by for example, liposome formation. Thus. in one embodiment, multimers of the invention, such as, for example, homodimers or homotrimers. are formed when polypeptides of the invention contact one another in solution. In another embodiment, heteromultimers of the invention, such as. for example, heterotrimers or heterotetramers, are formed when polypeptides of the invention contact antibodies to the polypeptides of the invention (including antibodies to the heterologous polypeptide sequence in a fusion protein of the invention) in solution. In other embodiments, multimers of the invention are formed by covalent associations with and/or between the polypeptides of the invention. Such covalent associations may involve one or more amino acid residues contained in the polypeptide sequence (e.g., that recited in SEQ ID NO:Y, or contained in a polypeptide encoded by SEQ ID NO:X, and/or by the cDNA in the related cDNA clone contained in a deposited library). In one instance, the covalent associations are cross-linking between cysteine residues located within the polypeptide sequences which interact in the native (i.e., naturally occurring) polypeptide. In another instance, the covalent associations are the consequence of chemical or recombinant manipulation. Alternatively, such covalent associations may involve one or more amino acid residues contained in the heterologous polypeptide sequence in a fusion protein. In one example, covalent associations are between the heterologous sequence contained in a fusion protein of the invention (see, e.g., US Patent Number 5,478,925). In a specific example, the covalent associations are between the heterologous sequence contained in a Fc fusion protein of the invention (as described herein). In another specific example, covalent associations of fusion proteins of the invention are between heterologous polypeptide sequence from another protein that is capable of forming covalently associated multimers, such as for

example, oseteoprotegerin (see, e.g., International Publication NO: WO 98/49305, the contents of which are herein incorporated by reference in its entirety). In another embodiment, two or more polypeptides of the invention are joined through peptide linkers. Examples include those peptide linkers described in U.S. Pat. No. 5,073,627 (hereby incorporated by reference). Proteins comprising multiple polypeptides of the invention separated by peptide linkers may be produced using conventional recombinant DNA technology.

Another method for preparing multimer polypeptides of the invention involves use of polypeptides of the invention fused to a leucine zipper or isoleucine zipper polypeptide sequence. Leucine zipper and isoleucine zipper domains are polypeptides that promote multimerization of the proteins in which they are found. Leucine zippers were originally identified in several DNA-binding proteins (Landschulz et al., Science 240:1759, (1988)), and have since been found in a variety of different proteins. Among the known leucine zippers are naturally occurring peptides and derivatives thereof that dimerize or trimerize. Examples of leucine zipper domains suitable for producing soluble multimeric proteins of the invention are those described in PCT application WO 94/10308, hereby incorporated by reference. Recombinant fusion proteins comprising a polypeptide of the invention fused to a polypeptide sequence that dimerizes or trimerizes in solution are expressed in suitable host cells, and the resulting soluble multimeric fusion protein is recovered from the culture supernatant using techniques known in the art.

Trimeric polypeptides of the invention may offer the advantage of enhanced biological activity. Preferred leucine zipper moieties and isoleucine moieties are those that preferentially form trimers. One example is a leucine zipper derived from lung surfactant protein D (SPD), as described in Hoppe et al. (FEBS Letters 344:191, (1994)) and in U.S. patent application Ser. No. 08/446,922, hereby incorporated by reference. Other peptides derived from naturally occurring trimeric proteins may be employed in preparing trimeric polypeptides of the invention.

In another example, proteins of the invention are associated by interactions between Flag® polypeptide sequence contained in fusion proteins of the invention

containing Flag® polypeptide seuqence. In a further embodiment, associations proteins of the invention are associated by interactions between heterologous polypeptide sequence contained in Flag® fusion proteins of the invention and anti-Flag® antibody.

The multimers of the invention may be generated using chemical techniques known in the art. For example, polypeptides desired to be contained in the multimers of the invention may be chemically cross-linked using linker molecules and linker molecule length optimization techniques known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, multimers of the invention may be generated using techniques known in the art to form one or more inter-molecule cross-links between the cysteine residues located within the sequence of the polypeptides desired to be contained in the multimer (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Further, polypeptides of the invention may be routinely modified by the addition of cysteine or biotin to the C-terminus or N-terminus of the polypeptide and techniques known in the art may be applied to generate multimers containing one or more of these modified polypeptides (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). Additionally, techniques known in the art may be applied to generate liposomes containing the polypeptide components desired to be contained in the multimer of the invention (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Alternatively, multimers of the invention may be generated using genetic engineering techniques known in the art. In one embodiment, polypeptides contained in multimers of the invention are produced recombinantly using fusion protein technology described herein or otherwise known in the art (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In a specific embodiment, polynucleotides coding for a homodimer of the invention are generated by ligating a polynucleotide sequence encoding a polypeptide of the invention to a sequence encoding a linker polypeptide and then further to a synthetic

polynucleotide encoding the translated product of the polypeptide in the reverse orientation from the original C-terminus to the N-terminus (lacking the leader sequence) (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety). In another embodiment, recombinant techniques described herein or otherwise known in the art are applied to generate recombinant polypeptides of the invention which contain a transmembrane domain (or hyrophobic or signal peptide) and which can be incorporated by membrane reconstitution techniques into liposomes (see, e.g., US Patent Number 5,478,925, which is herein incorporated by reference in its entirety).

Antibodies

Further polypeptides of the invention relate to antibodies and T-cell antigen receptors (TCR) which immunospecifically bind a polypeptide, polypeptide fragment, or variant of SEQ ID NO:Y, and/or an epitope, of the present invention (as determined by immunoassays well known in the art for assaying specific antibody-antigen binding). Antibodies of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), and epitope-binding fragments of any of the above. The term "antibody," as used herein, refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. The immunoglobulin molecules of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

Most preferably the antibodies are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')2, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody

fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. The antibodies of the invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, ship rabbit, goat, guinea pig, camel, horse, or chicken. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulin and that do not express endogenous immunoglobulins, as described infra and, for example in, U.S. Patent No. 5,939,598 by Kucherlapati et al.

The antibodies of the present invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a polypeptide of the present invention or may be specific for both a polypeptide of the present invention as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies of the present invention may be described or specified in terms of the epitope(s) or portion(s) of a polypeptide of the present invention which they recognize or specifically bind. The epitope(s) or polypeptide portion(s) may be specified as described herein, e.g., by N-terminal and C-terminal positions, or by size in contiguous amino acid residues. Antibodies which specifically bind any epitope or polypeptide of the present invention may also be excluded. Therefore, the present invention includes antibodies that specifically bind polypeptides of the present invention, and allows for the exclusion of the same.

Antibodies of the present invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog,

or homolog of a polypeptide of the present invention are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In specific embodiments, antibodies of the present invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a polypeptide of the present invention are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide of the present invention under stringent hybridization conditions (as described herein). Antibodies of the present invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6}M, 5×10^{-7} M, 10^{7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, $^{10-12}$ M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or $^{10-15}$ M.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of the invention as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein. In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies of the present invention may act as agonists or antagonists of the polypeptides of the present invention. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. Preferrably, antibodies of the present invention bind an antigenic epitope disclosed herein, or a portion thereof. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described supra). In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody.

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the peptides of the invention disclosed herein. The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res.

58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al.. Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties).

Antibodies of the present invention may be used, for example, but not limited to, to purify, detect, and target the polypeptides of the present invention, including both in vitro and in vivo diagnostic and therapeutic methods. For example, the antibodies have use in immunoassays for qualitatively and quantitatively measuring levels of the polypeptides of the present invention in biological samples. See, e.g., Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) (incorporated by reference herein in its entirety).

As discussed in more detail below, the antibodies of the present invention may be used either alone or in combination with other compositions. The antibodies may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, antibodies of the present invention may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. See, e.g., PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

The antibodies of the invention include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from generating an anti-idiotypic response. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphylation, amidation, derivatization by known protecting/blocking groups,

proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

The antibodies of the present invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of- interest can be produced by various procedures well known in the art. For example, a polypeptide of the invention can be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art and are discussed in detail in the Examples. In a non-limiting example, mice can be immunized with a polypeptide of the invention or a cell expressing such peptide. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire

or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999

(1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including in vivo use of antibodies in humans and in vitro detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5.807,715; 4,816,567; and 4,816397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering 7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody

libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825;

5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; and 5,939,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

Further, antibodies to the polypeptides of the invention can, in turn, be utilized to generate anti-idiotype antibodies that "mimic" polypeptides of the invention using techniques well known to those skilled in the art. (See, e.g., Greenspan & Bona, FASEB J. 7(5):437-444; (1989) and Nissinoff, J. Immunol. 147(8):2429-2438 (1991)). For example, antibodies which bind to and competitively inhibit polypeptide multimerization and/or binding of a polypeptide of the invention to a ligand can be used to generate anti-idiotypes that "mimic" the polypeptide multimerization and/or binding domain and, as a consequence, bind to and neutralize polypeptide and/or its ligand. Such neutralizing anti-idiotypes or Fab fragments of such anti-idiotypes can be used in therapeutic regimens to neutralize polypeptide ligand. For example, such anti-idiotypic antibodies can be used to bind a polypeptide of the invention and/or to bind its ligands/receptors, and thereby block its biological activity.

Polynucleotides Encoding Antibodies

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody of the invention and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined supra, to polynucleotides that encode an antibody, preferably, that specifically binds to a

polypeptide of the invention, preferably, an antibody that binds to a polypeptide having the amino acid sequence of SEQ ID NO:Y.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody of the invention) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley &

Sons, NY, which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described supra. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed supra, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described supra, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived

from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in E. coli may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

Methods of Producing Antibodies

The antibodies of the invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques.

Recombinant expression of an antibody of the invention, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody of the invention or a single chain antibody of the invention), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. The invention, thus, provides replicable vectors comprising a

nucleotide sequence encoding an antibody molecule of the invention. or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody of the invention. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody of the invention, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention in situ. These include but are not limited to microorganisms such as bacteria (e.g., E. coli, B. subtilis) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., Saccharomyces, Pichia) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS, CHO,

BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as Escherichia coli, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., Gene 45:101 (1986); Cockett et al., Bio/Technology 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the E. coli expression vector pUR278 (Ruther et al., EMBO J. 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, Nucleic Acids Res. 13:3101-3109 (1985); Van Heeke & Schuster, J. Biol. Chem. 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in

Spodoptera frugiperda cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by in vitro or in vivo recombination. Insertion in a non- essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript,

glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators. polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgprt- or aprt- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991);

Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215); and hygro, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.

The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

The present invention encompasses antibodies recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to a polypeptide (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention to generate fusion proteins. The fusion does not necessarily need to be direct, but may occur through linker sequences. The antibodies may be specific for antigens other than polypeptides (or portion thereof, preferably at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 amino acids of the polypeptide) of the present invention. For example, antibodies may be used to target the polypeptides of the present invention to particular cell types, either in vitro or in vivo, by fusing or conjugating the polypeptides of the present invention to antibodies specific for particular cell surface receptors. Antibodies fused or conjugated to the polypeptides of the present invention may also be used in in vitro immunoassays and purification methods using methods known in the art. See e.g., Harbor et al., supra, and PCT publication WO 93/21232; EP 439,095; Naramura et al., Immunol. Lett. 39:91-99 (1994); U.S. Patent 5,474,981; Gillies et al., PNAS 89:1428-1432 (1992); Fell et al., J. Immunol. 146:2446-2452(1991), which are incorporated by reference in their entireties.

The present invention further includes compositions comprising the polypeptides of the present invention fused or conjugated to antibody domains other than the variable regions. For example, the polypeptides of the present invention may be fused or conjugated to an antibody Fc region, or portion thereof. The antibody

portion fused to a polypeptide of the present invention may comprise the constant region, hinge region, CH1 domain, CH2 domain, and CH3 domain or any combination of whole domains or portions thereof. The polypeptides may also be fused or conjugated to the above antibody portions to form multimers. For example, Fc portions fused to the polypeptides of the present invention can form dimers through disulfide bonding between the Fc portions. Higher multimeric forms can be made by fusing the polypeptides to portions of IgA and IgM. Methods for fusing or conjugating the polypeptides of the present invention to antibody portions are known in the art. See, e.g., U.S. Patent Nos. 5,336,603; 5,622,929; 5,359,046; 5,349,053; 5,447,851; 5,112,946; EP 307,434; EP 367,166; PCT publications WO 96/04388; WO 91/06570; Ashkenazi et al., Proc. Natl. Acad. Sci. USA 88:10535-10539 (1991); Zheng et al., J. Immunol. 154:5590-5600 (1995); and Vil et al., Proc. Natl. Acad. Sci. USA 89:11337- 11341(1992) (said references incorporated by reference in their entireties).

As discussed, supra, the polypeptides corresponding to a polypeptide, polypeptide fragment, or a variant of SEQ ID NO:Y may be fused or conjugated to the above antibody portions to increase the in vivo half life of the polypeptides or for use in immunoassays using methods known in the art. Further, the polypeptides corresponding to SEQ ID NO:Y may be fused or conjugated to the above antibody portions to facilitate purification. One reported example describes chimeric proteins consisting of the first two domains of the human CD4-polypeptide and various domains of the constant regions of the heavy or light chains of mammalian immunoglobulins. (EP 394,827; Traunecker et al., Nature 331:84-86 (1988). The polypeptides of the present invention fused or conjugated to an antibody having disulfide- linked dimeric structures (due to the IgG) may also be more efficient in binding and neutralizing other molecules, than the monomeric secreted protein or protein fragment alone. (Fountoulakis et al., J. Biochem. 270:3958-3964 (1995)). In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, for example, improved pharmacokinetic properties. (EP A 232,262). Alternatively, deleting the Fc part after the fusion protein has been

expressed, detected, and purified, would be desired. For example, the Fc portion may hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, for example, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. (See, Bennett et al., J. Molecular Recognition 8:52-58 (1995); Johanson et al., J. Biol. Chem. 270:9459-9471 (1995).

Moreover, the antibodies or fragments thereof of the present invention can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish

peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include 125I, 131I, 111In or 99Tc.

Further, an antibody or fragment thereof may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, 213Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclothosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria

toxin; a protein such as tumor necrosis factor, a-interferon, ß-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al., Int. Immunol.*, *6*:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev. 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

Immunophenotyping

The antibodies of the invention may be utilized for immunophenotyping of cell lines and biological samples. The translation product of the gene of the present invention may be useful as a cell specific marker, or more specifically as a cellular marker that is differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 5,985,660; and Morrison *et al.*, *Cell*, *96*:737-49 (1999)).

These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

Assays For Antibody Binding

The antibodies of the invention may be assayed for immunospecific binding by any method known in the art. The immunoassays which can be used include but are not limited to competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays,

complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X- 100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding the antibody of interest to the cell lysate, incubating for a period of time (e.g., 1-4 hours) at 4° C, adding protein A and/or protein G sepharose beads to the cell lysate, incubating for about an hour or more at 4° C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody of interest to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the antibody to an antigen and decrease the background (e.g., pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (e.g., 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (e.g., PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (e.g., PBS-Tween 20), blocking the membrane with primary antibody (the antibody of interest) diluted in blocking buffer, washing the membrane in washing buffer, blocking the membrane with a secondary antibody (which recognizes the primary antibody, e.g., an anti-human

antibody) conjugated to an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) or radioactive molecule (e.g., 32P or 125I) diluted in blocking buffer. washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96 well microtiter plate with the antigen, adding the antibody of interest conjugated to a detectable compound such as an enzymatic substrate (e.g., horseradish peroxidase or alkaline phosphatase) to the well and incubating for a period of time, and detecting the presence of the antigen. In ELISAs the antibody of interest does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody of interest) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the antibody may be coated to the well. In this case, a second antibody conjugated to a detectable compound may be added following the addition of the antigen of interest to the coated well. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an antibody to an antigen and the off-rate of an antibody-antigen interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (e.g., 3H or 125I) with the antibody of interest in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody of interest for a particular antigen and the binding off-rates can be determined from the data by

scatchard plot analysis. Competition with a second antibody can also be determined using radioimmunoassays. In this case, the antigen is incubated with antibody of interest conjugated to a labeled compound (e.g., 3H or 125I) in the presence of increasing amounts of an unlabeled second antibody.

Therapeutic Uses

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein) and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a polypeptide of the invention, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a polypeptide of the invention includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. Antibodies of the invention may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of the present invention for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

The antibodies of the invention may be administered alone or in combination with other types of treatments (e.g., radiation therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, and 10^{-15} M.

Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies or functional derivatives thereof, are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic

acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described below.

For general reviews of the methods of gene therapy, see Goldspiel et al., Clinical Pharmacy 12:488-505 (1993); Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, TIBTECH 11(5):155-215 (1993). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons. NY (1993); and Kriegler, Gene Transfer and Expression. A Laboratory Manual, Stockton Press, NY (1990).

In a preferred aspect, the compound comprises nucleic acid sequences encoding an antibody, said nucleic acid sequences being part of expression vectors that express the antibody or fragments or chimeric proteins or heavy or light chains thereof in a suitable host. In particular, such nucleic acid sequences have promoters operably linked to the antibody coding region, said promoter being inducible or constitutive, and, optionally, tissue-specific. In another particular embodiment, nucleic acid molecules are used in which the antibody coding sequences and any other desired sequences are flanked by regions that promote homologous recombination at a desired site in the genome, thus providing for intrachromosomal expression of the antibody encoding nucleic acids (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989). In specific embodiments, the expressed antibody molecule is a single chain antibody; alternatively, the nucleic acid sequences include sequences encoding both the heavy and light chains, or fragments thereof, of the antibody.

Delivery of the nucleic acids into a patient may be either direct, in which case the patient is directly exposed to the nucleic acid or nucleic acid- carrying vectors, or indirect, in which case, cells are first transformed with the nucleic acids in vitro, then

transplanted into the patient. These two approaches are known, respectively, as in vivo or ex vivo gene therapy.

In a specific embodiment, the nucleic acid sequences are directly administered in vivo, where it is expressed to produce the encoded product. This can be accomplished by any of numerous methods known in the art, e.g., by constructing them as part of an appropriate nucleic acid expression vector and administering it so that they become intracellular, e.g., by infection using defective or attenuated retrovirals or other viral vectors (see U.S. Patent No. 4,980,286), or by direct injection of naked DNA, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents. encapsulation in liposomes, microparticles, or microcapsules, or by administering them in linkage to a peptide which is known to enter the nucleus, by administering it in linkage to a ligand subject to receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)) (which can be used to target cell types specifically expressing the receptors), etc. In another embodiment, nucleic acid-ligand complexes can be formed in which the ligand comprises a fusogenic viral peptide to disrupt endosomes, allowing the nucleic acid to avoid lysosomal degradation. In yet another embodiment, the nucleic acid can be targeted in vivo for cell specific uptake and expression, by targeting a specific receptor (see, e.g., PCT Publications WO 92/06180; WO 92/22635; WO92/20316; WO93/14188, WO 93/20221). Alternatively, the nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination (Koller and Smithies, Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); Zijlstra et al., Nature 342:435-438 (1989)).

In a specific embodiment, viral vectors that contains nucleic acid sequences encoding an antibody of the invention are used. For example, a retroviral vector can be used (see Miller et al., Meth. Enzymol. 217:581-599 (1993)). These retroviral vectors contain the components necessary for the correct packaging of the viral genome and integration into the host cell DNA. The nucleic acid sequences encoding the antibody to be used in gene therapy are cloned into one or more vectors, which

facilitates delivery of the gene into a patient. More detail about retroviral vectors can be found in Boesen et al., Biotherapy 6:291-302 (1994), which describes the use of a retroviral vector to deliver the mdr1 gene to hematopoietic stem cells in order to make the stem cells more resistant to chemotherapy. Other references illustrating the use of retroviral vectors in gene therapy are: Clowes et al., J. Clin. Invest. 93:644-651 (1994); Kiem et al., Blood 83:1467-1473 (1994); Salmons and Gunzberg, Human Gene Therapy 4:129-141 (1993); and Grossman and Wilson, Curr. Opin. in Genetics and Devel. 3:110-114 (1993).

Adenoviruses are other viral vectors that can be used in gene therapy. Adenoviruses are especially attractive vehicles for delivering genes to respiratory epithelia. Adenoviruses naturally infect respiratory epithelia where they cause a mild disease. Other targets for adenovirus-based delivery systems are liver, the central nervous system, endothelial cells, and muscle. Adenoviruses have the advantage of being capable of infecting non-dividing cells. Kozarsky and Wilson, Current Opinion in Genetics and Development 3:499-503 (1993) present a review of adenovirus-based gene therapy. Bout et al., Human Gene Therapy 5:3-10 (1994) demonstrated the use of adenovirus vectors to transfer genes to the respiratory epithelia of rhesus monkeys. Other instances of the use of adenoviruses in gene therapy can be found in Rosenfeld et al., Science 252:431-434 (1991); Rosenfeld et al., Cell 68:143- 155 (1992); Mastrangeli et al., J. Clin. Invest. 91:225-234 (1993); PCT Publication WO94/12649; and Wang, et al., Gene Therapy 2:775-783 (1995). In a preferred embodiment, adenovirus vectors are used.

Adeno-associated virus (AAV) has also been proposed for use in gene therapy (Walsh et al., Proc. Soc. Exp. Biol. Med. 204:289-300 (1993); U.S. Patent No. 5,436,146).

Another approach to gene therapy involves transferring a gene to cells in tissue culture by such methods as electroporation, lipofection, calcium phosphate mediated transfection, or viral infection. Usually, the method of transfer includes the transfer of a selectable marker to the cells. The cells are then placed under selection

to isolate those cells that have taken up and are expressing the transferred gene. Those cells are then delivered to a patient.

In this embodiment, the nucleic acid is introduced into a cell prior to administration in vivo of the resulting recombinant cell. Such introduction can be carried out by any method known in the art, including but not limited to transfection, electroporation, microinjection, infection with a viral or bacteriophage vector containing the nucleic acid sequences, cell fusion, chromosome-mediated gene transfer, microcell-mediated gene transfer, spheroplast fusion, etc. Numerous techniques are known in the art for the introduction of foreign genes into cells (see, e.g., Loeffler and Behr, Meth. Enzymol. 217:599-618 (1993); Cohen et al., Meth. Enzymol. 217:618-644 (1993); Cline, Pharmac. Ther. 29:69-92m (1985) and may be used in accordance with the present invention, provided that the necessary developmental and physiological functions of the recipient cells are not disrupted. The technique should provide for the stable transfer of the nucleic acid to the cell, so that the nucleic acid is expressible by the cell and preferably heritable and expressible by its cell progeny.

The resulting recombinant cells can be delivered to a patient by various methods known in the art. Recombinant blood cells (e.g., hematopoietic stem or progenitor cells) are preferably administered intravenously. The amount of cells envisioned for use depends on the desired effect, patient state, etc., and can be determined by one skilled in the art.

Cells into which a nucleic acid can be introduced for purposes of gene therapy encompass any desired, available cell type, and include but are not limited to epithelial cells, endothelial cells, keratinocytes, fibroblasts, muscle cells, hepatocytes; blood cells such as Tlymphocytes, Blymphocytes, monocytes, macrophages, neutrophils, eosinophils, megakaryocytes, granulocytes; various stem or progenitor cells, in particular hematopoietic stem or progenitor cells, e.g., as obtained from bone marrow, umbilical cord blood, peripheral blood, fetal liver, etc.

In a preferred embodiment, the cell used for gene therapy is autologous to the patient.

In an embodiment in which recombinant cells are used in gene therapy, nucleic acid sequences encoding an antibody are introduced into the cells such that they are expressible by the cells or their progeny, and the recombinant cells are then administered in vivo for therapeutic effect. In a specific embodiment, stem or progenitor cells are used. Any stem and/or progenitor cells which can be isolated and maintained in vitro can potentially be used in accordance with this embodiment of the present invention (see e.g. PCT Publication WO 94/08598; Stemple and Anderson, Cell 71:973-985 (1992); Rheinwald, Meth. Cell Bio. 21A:229 (1980); and Pittelkow and Scott, Mayo Clinic Proc. 61:771 (1986)).

In a specific embodiment, the nucleic acid to be introduced for purposes of gene therapy comprises an inducible promoter operably linked to the coding region, such that expression of the nucleic acid is controllable by controlling the presence or absence of the appropriate inducer of transcription. Demonstration of Therapeutic or Prophylactic Activity

The compounds or pharmaceutical compositions of the invention are preferably tested in vitro, and then in vivo for the desired therapeutic or prophylactic activity, prior to use in humans. For example, in vitro assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, in vitro assays which can be used to determine whether administration of a specific compound is indicated, include in vitro cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

Therapeutic/Prophylactic Administration and Composition

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical

composition of the invention, preferably a polypeptide or antibody of the invention. In a preferred aspect, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after

surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, Science 249:1527-1533 (1990); Treat et al., in Liposomes in the Therapy of Infectious Disease and Cancer, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, ibid., pp. 317-327; see generally ibid.)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, supra; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)). In another embodiment, polymeric materials can be used (see Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, i.e., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered in vivo to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by

use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox- like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable

pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a polypeptide of the invention can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of

the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies. the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

Diagnosis and Imaging

Labeled antibodies, and derivatives and analogs thereof, which specifically bind to a polypeptide of interest can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of a polypeptide of the invention. The invention provides for the detection of aberrant expression of a polypeptide of interest, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level,

whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of aberrant expression.

The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the polypeptide of interest in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed polypeptide gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell . Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One aspect of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a polypeptide of interest in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval

following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the polypeptide is expressed (and for unbound labeled molecule to be cleared to background level); c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the polypeptide of interest. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of 99mTc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which contain the specific protein. In vivo tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for in vivo scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al., U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patent using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a patient using magnetic resonance imaging (MRI).

Kits

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody of the invention, preferably a purified antibody, in one or more containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention. the kit is a diagnostic kit for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments. the kit may include a recombinantly produced or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment. the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens. and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment. the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of

bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface- bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

Uses of the Polynucleotides

Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

The pancreatic cancer antigen polynucleotides of the present invention are useful for chromosome identification. There exists an ongoing need to identify new chromosome markers, since few chromosome marking reagents, based on actual sequence data (repeat polymorphisms), are presently available. Each sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome, thus each polynucleotide of the present invention can routinely be used as a chromosome marker using techniques known in the art.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably at least 15 bp (e.g., 15-25 bp) from the sequences shown in SEQ ID NO:X, or the complement thereto. Primers can optionally be selected using computer analysis so that primers do not span more than one predicted exon in the genomic DNA. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to SEQ ID NO:X will yield an amplified fragment.

Similarly, somatic hybrids provide a rapid method of PCR mapping the polynucleotides to particular chromosomes. Three or more clones can be assigned per day using a single thermal cycler. Moreover, sublocalization of the polynucleotides can be achieved with panels of specific chromosome fragments. Other gene mapping strategies that can be used include in situ hybridization, prescreening with labeled flow-sorted chromosomes, preselection by hybridization to construct chromosome specific-cDNA libraries, and computer mapping techniques (See, e.g., Shuler, Trends Biotechnol 16:456-459 (1998) which is hereby incorporated by reference in its entirety).

Precise chromosomal location of the polynucleotides can also be achieved using fluorescence in situ hybridization (FISH) of a metaphase chromosomal spread. This technique uses polynucleotides as short as 500 or 600 bases; however, polynucleotides 2,000-4,000 bp are preferred. For a review of this technique, see Verma et al., "Human Chromosomes: a Manual of Basic Techniques," Pergamon Press, New York (1988).

For chromosome mapping, the polynucleotides can be used individually (to mark a single chromosome or a single site on that chromosome) or in panels (for marking multiple sites and/or multiple chromosomes).

Thus, the present invention also provides a method for chromosomal localization which involves (a) preparing PCR primers from the polynucleotide sequences in Table 3 and SEQ ID NO:X and (b) screening somatic cell hybrids containing individual chromosomes.

The polynucleotides of the present invention would likewise be useful for radiation hybrid mapping, HAPPY mapping, and long range restriction mapping. For a review of these techniques and others known in the art, see, e.g. Dear, "Genome Mapping: A Practical Approach," IRL Press at Oxford University Press, London (1997); Aydin, J. Mol. Med. 77:691-694 (1999); Hacia et al., Mol. Psychiatry 3:483-492 (1998); Herrick et al., Chromosome Res. 7:409-423 (1999); Hamilton et al., Methods Cell Biol. 62:265-280 (2000); and/or Ott, J. Hered. 90:68-70 (1999) each of which is hereby incorporated by reference in its entirety.

Once a polynucleotide has been mapped to a precise chromosomal location, the physical position of the polynucleotide can be used in linkage analysis. Linkage analysis establishes coinheritance between a chromosomal location and presentation of a particular disease. (Disease mapping data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library).) Assuming 1 megabase mapping resolution and one gene per 20 kb, a cDNA precisely localized to a chromosomal region associated with the disease could be one of 50-500 potential causative genes.

Thus, once coinheritance is established, differences in a polynucleotide of the invention and the corresponding gene between affected and unaffected individuals can be examined. First, visible structural alterations in the chromosomes, such as deletions or translocations, are examined in chromosome spreads or by PCR. If no structural alterations exist, the presence of point mutations are ascertained. Mutations observed in some or all affected individuals, but not in normal individuals, indicates that the mutation may cause the disease. However, complete sequencing of the polypeptide and the corresponding gene from several normal individuals is required to distinguish the mutation from a polymorphism. If a new polymorphism is identified, this polymorphic polypeptide can be used for further linkage analysis.

Furthermore, increased or decreased expression of the gene in affected individuals as compared to unaffected individuals can be assessed using the polynucleotides of the invention. Any of these alterations (altered expression,

chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention provides a method of detecting increased or decreased expression levels of the pancreatic cancer polynucleotides in affected individuals as compared to unaffected individuals using polynucleotides of the present invention and techniques known in the art, including but not limited to the method described in Example 11. Any of these alterations (altered expression, chromosomal rearrangement, or mutation) can be used as a diagnostic or prognostic marker.

Thus, the invention also provides a diagnostic method useful during diagnosis of a pancreas related disorder, including pancreas cancer, involving measuring the expression level of pancreatic cancer polynucleotides in pancreatic tissue or other cells or body fluid from an individual and comparing the measured gene expression level with a standard pancreatic cancer polynucleotide expression level, whereby an increase or decrease in the gene expression level compared to the standard is indicative of a pancreas related disorder.

In still another embodiment, the invention includes a kit for analyzing samples for the presence of proliferative and/or cancerous polynucleotides derived from a test subject. In a general embodiment, the kit includes at least one polynucleotide probe containing a nucleotide sequence that will specifically hybridize with a polynucleotide of the invention and a suitable container. In a specific embodiment, the kit includes two polynucleotide probes defining an internal region of the polynucleotide of the invention, where each probe has one strand containing a 31'mer-end internal to the region. In a further embodiment, the probes may be useful as primers for polymerase chain reaction amplification.

Where a diagnosis of a pancreas related disorder, including, for example, diagnosis of a tumor, has already been made according to conventional methods, the present invention is useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed pancreatic cancer polynucleotide expression will experience a worse clinical outcome relative to patients expressing the gene at a level nearer the standard level.

By "measuring the expression level of pancreatic cancer polynucleotides" is intended qualitatively or quantitatively measuring or estimating the level of the pancreatic cancer polypeptide or the level of the mRNA encoding the pancreatic cancer polypeptide in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the pancreatic cancer polypeptide level or mRNA level in a second biological sample). Preferably, the pancreatic cancer polypeptide level or mRNA level in the first biological sample is measured or estimated and compared to a standard pancreatic cancer polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the pancreas related disorder or being determined by averaging levels from a population of individuals not having a pancreas related disorder. As will be appreciated in the art, once a standard pancreatic cancer polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, body fluid, cell line, tissue culture, or other source which contains pancreatic cancer polypeptide or the corresponding mRNA. As indicated, biological samples include body fluids (such as bile, lymph, sera, plasma, urine, synovial fluid and spinal fluid) which contain the pancreatic cancer polypeptide, pancreas tissue, and other tissue sources found to express the pancreatic cancer polypeptide. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

The method(s) provided above may preferrably be applied in a diagnostic method and/or kits in which polynucleotides and/or polypeptides of the invention are attached to a solid support. In one exemplary method, the support may be a "gene chip" or a "biological chip" as described in US Patents 5,837,832, 5,874,219, and 5,856,174. Further, such a gene chip with pancreatic cancer polynucleotides attached may be used to identify polymorphisms between the pancreatic cancer polynucleotide sequences, with polynucleotides isolated from a test subject. The knowledge of such

polymorphisms (i.e. their location, as well as, their existence) would be beneficial in identifying disease loci for many disorders, such as for example, in neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders. pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders. and/or cancerous diseases and conditions, though most preferably in pancreas related proliferative, and/or cancerous diseases and conditions. Such a method is described in US Patents 5,858,659 and 5,856,104. The US Patents referenced supra are hereby incorporated by reference in their entirety herein.

The present invention encompasses pancreatic cancer polynucleotides that are chemically synthesized, or reproduced as peptide nucleic acids (PNA), or according to other methods known in the art. The use of PNAs would serve as the preferred form if the polynucleotides of the invention are incorporated onto a solid support, or gene chip. For the purposes of the present invention, a peptide nucleic acid (PNA) is a polyamide type of DNA analog and the monomeric units for adenine, guanine, thymine and cytosine are available commercially (Perceptive Biosystems). Certain components of DNA, such as phosphorus, phosphorus oxides, or deoxyribose derivatives, are not present in PNAs. As disclosed by P. E. Nielsen, M. Egholm, R. H. Berg and O. Buchardt, Science 254, 1497 (1991); and M. Egholm. O. Buchardt, L.Christensen, C. Behrens, S. M. Freier, D. A. Driver, R. H. Berg, S. K. Kim, B. Norden. and P. E. Nielsen, Nature 365. 666 (1993), PNAs bind specifically and tightly to complementary DNA strands and are not degraded by nucleases. In fact, PNA binds more strongly to DNA than DNA itself does. This is probably because there is no electrostatic repulsion between the two strands, and also the polyamide backbone is more flexible. Because of this, PNA/DNA duplexes bind under a wider range of stringency conditions than DNA/DNA duplexes, making it easier to perform multiplex hybridization. Smaller probes can be used than with DNA due to the strong binding. In addition, it is more likely that single base mismatches can be determined with PNA/DNA hybridization because a single mismatch in a PNA/DNA 15-mer lowers the melting point (T.sub.m) by 8°-20° C, vs. 4°-16° C for the DNA/DNA 15-mer duplex. Also, the absence of charge groups in PNA means that hybridization can

be done at low ionic strengths and reduce possible interference by salt during the analysis.

The present invention have uses which include, but are not limited to, detecting cancer in mammals. In particular the invention is useful during diagnosis of pathological cell proliferative neoplasias which include, but are not limited to: acute myelogenous leukemias including acute monocytic leukemia, acute myeloblastic leukemia, acute promyelocytic leukemia, acute myelomonocytic leukemia, acute erythroleukemia, acute megakaryocytic leukemia, and acute undifferentiated leukemia, etc.; and chronic myelogenous leukemias including chronic myelomonocytic leukemia, chronic granulocytic leukemia, etc. Preferred mammals include monkeys, apes, cats, dogs, cows, pigs, horses, rabbits and humans. Particularly preferred are humans.

Pathological cell proliferative disorders are often associated with inappropriate activation of proto-oncogenes. (Gelmann, E. P. et al., "The Etiology of Acute Leukemia: Molecular Genetics and Viral Oncology," in Neoplastic Diseases of the Blood, Vol 1., Wiernik, P. H. et al. eds., 161-182 (1985)). Neoplasias are now believed to result from the qualitative alteration of a normal cellular gene product, or from the quantitative modification of gene expression by insertion into the chromosome of a viral sequence, by chromosomal translocation of a gene to a more actively transcribed region, or by some other mechanism. (Gelmann et al., supra) It is likely that mutated or altered expression of specific genes is involved in the pathogenesis of some leukemias, among other tissues and cell types. (Gelmann et al., supra) Indeed, the human counterparts of the oncogenes involved in some animal neoplasias have been amplified or translocated in some cases of human leukemia and carcinoma. (Gelmann et al., supra)

For example, c-myc expression is highly amplified in the non-lymphocytic leukemia cell line HL-60. When HL-60 cells are chemically induced to stop proliferation, the level of c-myc is found to be downregulated. (International Publication Number WO 91/15580). However, it has been shown that exposure of HL-60 cells to a DNA construct that is complementary to the 5' end of c-myc or c-

myb blocks translation of the corresponding mRNAs which downregulates expression of the c-myc or c-myb proteins and causes arrest of cell proliferation and differentiation of the treated cells. (International Publication Number WO 91/15580; Wickstrom et al., Proc. Natl. Acad. Sci. 85:1028 (1988); Anfossi et al., Proc. Natl. Acad. Sci. 86:3379 (1989)). However, the skilled artisan would appreciate the present invention's usefulness is not limited to treatment of proliferative disorders of hematopoietic cells and tissues, in light of the numerous cells and cell types of varying origins which are known to exhibit proliferative phenotypes.

In addition to the foregoing, a pancreatic cancer antigen polynucleotide can be used to control gene expression through triple helix formation or through antisense DNA or RNA. Antisense techniques are discussed, for example, in Okano, J. Neurochem. 56: 560 (1991); "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance Lee et al., Nucleic Acids Research 6: 3073 (1979); Cooney et al., Science 241: 456 (1988); and Dervan et al., Science 251: 1360 (1991). Both methods rely on binding of the polynucleotide to a complementary DNA or RNA. For these techniques, preferred polynucleotides are usually oligonucleotides 20 to 40 bases in length and complementary to either the region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res. 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1360 (1991)) or to the mRNA itself (antisense - Okano, J. Neurochem. 56:560 (1991); Oligodeoxy-nucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988).) Triple helix formation optimally results in a shut-off of RNA transcription from DNA, while antisense RNA hybridization blocks translation of an mRNA molecule into polypeptide. The oligonucleotide described above can also be delivered to cells such that the antisense RNA or DNA may be expressed in vivo to inhibit production of polypeptide of the present invention antigens. Both techniques are effective in model systems, and the information disclosed herein can be used to design antisense or triple helix polynucleotides in an effort to treat disease, and in particular, for the treatment of proliferative diseases and/or conditions.

Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell.

The polynucleotides are also useful for identifying individuals from minute biological samples. The United States military, for example, is considering the use of restriction fragment length polymorphism (RFLP) for identification of its personnel. In this technique, an individual's genomic DNA is digested with one or more restriction enzymes, and probed on a Southern blot to yield unique bands for identifying personnel. This method does not suffer from the current limitations of "Dog Tags" which can be lost, switched, or stolen, making positive identification difficult. The polynucleotides of the present invention can be used as additional DNA markers for RFLP.

The polynucleotides of the present invention can also be used as an alternative to RFLP, by determining the actual base-by-base DNA sequence of selected portions of an individual's genome. These sequences can be used to prepare PCR primers for amplifying and isolating such selected DNA, which can then be sequenced. Using this technique, individuals can be identified because each individual will have a unique set of DNA sequences. Once an unique ID database is established for an individual, positive identification of that individual, living or dead, can be made from extremely small tissue samples.

Forensic biology also benefits from using DNA-based identification techniques as disclosed herein. DNA sequences taken from very small biological samples such as tissues, e.g., hair or skin, or body fluids, e.g., blood, saliva, semen, synovial fluid, amniotic fluid, breast milk, lymph, pulmonary sputum or surfactant, urine, fecal matter, etc., can be amplified using PCR. In one prior art technique, gene sequences amplified from polymorphic loci, such as DQa class II HLA gene, are used in forensic biology to identify individuals. (Erlich, H., PCR Technology, Freeman

and Co. (1992).) Once these specific polymorphic loci are amplified, they are digested with one or more restriction enzymes, yielding an identifying set of bands on a Southern blot probed with DNA corresponding to the DQa class II HLA gene. Similarly, polynucleotides of the present invention can be used as polymorphic markers for forensic purposes.

There is also a need for reagents capable of identifying the source of a particular tissue. Such need arises, for example, in forensics when presented with tissue of unknown origin. Appropriate reagents can comprise, for example, DNA probes or primers specific to pancreas or pancreatic cancer polynucleotides prepared from the sequences of the present invention. Panels of such reagents can identify tissue by species and/or by organ type. In a similar fashion, these reagents can be used to screen tissue cultures for contamination.

The polynucleotides of the present invention are also useful as hybridization probes for differential identification of the tissue(s) or cell type(s) present in a biological sample. Similarly, polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays) or cell type(s) (e.g., immunocytochemistry assays). In addition, for a number of disorders of the above tissues or cells, significantly higher or lower levels of gene expression of the polynucleotides/polypeptides of the present invention may be detected in certain tissues (e.g., tissues expressing polypeptides and/or polynucleotides of the present invention, pancreas and pancreatic cancer tissues and/or cancerous and/or wounded tissues) or bodily fluids (e.g., serum, plasma, urine, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, i.e., the expression level in healthy tissue from an individual not having the disorder.

Thus, the invention provides a diagnostic method of a disorder, which involves: (a) assaying gene expression level in cells or body fluid of an individual; (b) comparing the gene expression level with a standard gene expression level, whereby

an increase or decrease in the assayed gene expression level compared to the standard expression level is indicative of a disorder.

In the very least, the polynucleotides of the present invention can be used as molecular weight markers on Southern gels, as diagnostic probes for the presence of a specific mRNA in a particular cell type, as a probe to "subtract-out" known sequences in the process of discovering novel polynucleotides, for selecting and making oligomers for attachment to a "gene chip" or other support, to raise anti-DNA antibodies using DNA immunization techniques, and as an antigen to elicit an immune response.

<u>Uses of the Polypeptides</u>

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Polypeptides and antibodies directed to polypeptides of the present invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Antibodies can be used to assay levels of polypeptides encoded by polynucleotides of the invention in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd. ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru;

luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

In addition to assaying levels of polypeptide of the present invention in a biological sample, proteins can also be detected in vivo by imaging. Antibody labels or markers for in vivo imaging of protein include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the antibody by labeling of nutrients for the relevant hybridoma.

A protein-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example, ^{131}I, ^{112}In, ^{99m}Tc, (^{131}I, ^{125}I, ^{123}I, ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{115m}In, ^{113m}In, ^{112}In, ^{111}In), and technetium (^{99}Tc, ^{99m}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F, ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99m}Tc. The labeled antibody or antibody fragment will then preferentially accumulate at the location of cells which express the polypeptide encoded by a polynucleotide of the invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (e.g., polypeptides encoded by polynucleotides of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art. compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, ^{213}Bi, or other radioisotopes such as, for example, ^{103}Pd, ^{133}Xe, ^{131}I, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{35}S, ^{90}Y, ^{153}Sm, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, ^{90}Yttrium, ^{117}Tin, ^{186}Rhenium, ^{166}Holmium, and ^{188}Rhenium; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Techniques known in the art may be applied to label polypeptides of the invention (including antibodies). Such techniques include, but are not limited to, the

use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5.714,631; 5,696,239: 5,652,361; 5,505,931; 5,489.425; 5,435,990: 5,428,139: 5,342,604; 5,274,119; 4,994,560: and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a pancreatic cancer polypeptide of the present invention in cells or body fluid of an individual, or more preferrably, assaying the expression level of a pancreatic cancer polypeptide of the present invention in pancreatic cells or bile of an individual; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, pancreatic cancer antigen polypeptides of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions, preferably proliferative disorders of the pancreas, and/or cancerous disease and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor supressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a

membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

Similarly, antibodies directed to a polypeptide of the present invention can also be used to treat disease (as described supra, and elsewhere herein). For example, administration of an antibody directed to a polypeptide of the present invention can bind, and/or neutralize the polypeptide, and/or reduce overproduction of the polypeptide. Similarly, administration of an antibody can activate the polypeptide, such as by binding to a polypeptide bound to a membrane (receptor).

At the very least, the polypeptides of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art. Polypeptides can also be used to raise antibodies, which in turn are used to measure protein expression from a recombinant cell, as a way of assessing transformation of the host cell. Moreover, the polypeptides of the present invention can be used to test the following biological activities.

Gene Therapy Methods

Another aspect of the present invention is to gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of the polypeptide of the present invention. This method requires a polynucleotide which codes for a polypeptide of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a

polynucleotide of the present invention ex vivo, with the engineered cells then being provided to a patient to be treated with the polypeptide of the present invention. Such methods are well-known in the art. For example, see Belldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, the polynucleotide of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotide of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO,

pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1. and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the polynucleotide of the present invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the

tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. In vivo muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge

complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

Cationic liposomes are readily available. For example, N[1-2,3-dioleyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL, Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphoshatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the art. See, e.g., Straubinger et al., Methods of Immunology (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include Ca^{2+}-EDTA chelation (Papahadjopoulos et al., Biochim. Biophys. Acta

(1975) 394:483; Wilson et al., Cell (1979) 17:77); ether injection (Deamer, D. and Bangham, A., Biochim. Biophys. Acta (1976) 443:629; Ostro et al., Biochem. Biophys. Res. Commun. (1977) 76:836; Fraley et al., Proc. Natl. Acad. Sci. USA (1979) 76:3348); detergent dialysis (Enoch, H. and Strittmatter, P., Proc. Natl. Acad. Sci. USA (1979) 76:145); and reverse-phase evaporation (REV) (Fraley et al., J. Biol. Chem. (1980) 255:10431; Szoka, F. and Papahadjopoulos, D., Proc. Natl. Acad. Sci. USA (1978) 75:145; Schaefer-Ridder et al., Science (1982) 215:166), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide methods for delivering DNA-cationic lipid complexes to mammals.

In certain embodiments, cells are engineered, ex vivo or in vivo, using a retroviral particle containing RNA which comprises a sequence encoding a polypeptide of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-

19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a polypeptide of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either in vitro or in vivo. The transduced eukaryotic cells will express a polypeptide of the present invention.

In certain other embodiments, cells are engineered, ex vivo or in vivo, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses a polypeptide of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz, A. R. et al. (1974) Am. Rev. Respir. Dis.109:233-238). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155). Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature

365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or in vivo, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses,

cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either ex vivo or in vivo. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a polypeptide of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

Preferably, the polynucleotide encoding a polypeptide of the present invention contains a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery. For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, a recombinant molecule of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

Therapeutic compositions useful in systemic administration, include recombinant molecules of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment and its severity, and the route of administration. The

frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

Therapeutic compositions of the present invention can be administered to any animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

Biological Activities

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, can be used in assays to test for one or more biological activities. If these polynucleotides or polypeptides, or agonists or antagonists of the present invention, do exhibit activity in a particular assay, it is likely that these molecules may be involved in the diseases associated with the biological activity. Thus, the polynucleotides and polypeptides, and agonists or antagonists could be used to treat the associated disease.

Immune Activity

A polypeptide or polynucleotide, or agonists or antagonists of the present invention may be useful in treating deficiencies or disorders of the immune system, by activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune deficiencies or disorders may be genetic, somatic, such as cancer or some autoimmune disorders, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used as a marker or detector of a particular immune system disease or disorder.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may be useful in treating or detecting deficiencies or disorders of hematopoietic cells. Polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat those disorders associated with a decrease in certain (or many) types hematopoietic cells. Examples of immunologic deficiency syndromes include, but are not limited to: blood protein disorders (e.g. agammaglobulinemia, dysgammaglobulinemia), ataxia telangiectasia, common variable immunodeficiency, Digeorge Syndrome, HIV infection, HTLV-BLV infection, leukocyte adhesion deficiency syndrome, lymphopenia, phagocyte bactericidal dysfunction, severe combined immunodeficiency (SCIDs), Wiskott-Aldrich Disorder, anemia, thrombocytopenia, or hemoglobinuria.

Moreover, polynucleotides or polypeptides, or agonists or antagonists of the present invention could also be used to modulate hemostatic (the stopping of bleeding) or thrombolytic activity (clot formation). For example, by increasing hemostatic or thrombolytic activity, polynucleotides or polypeptides, or agonists or antagonists of the present invention could be used to treat blood coagulation disorders (e.g., afibrinogenemia, factor deficiencies), blood platelet disorders (e.g. thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, polynucleotides or polypeptides, or agonists or antagonists of the present invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment of heart attacks (infarction), strokes, or scarring.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be useful in treating or detecting autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention

that can inhibit an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Examples of autoimmune disorders that can be treated or detected include, but are not limited to: Addison's Disease, hemolytic anemia, antiphospholipid syndrome, rheumatoid arthritis, dermatitis, allergic encephalomyelitis, glomerulonephritis, Goodpasture's Syndrome, Graves' Disease, Multiple Sclerosis, Myasthenia Gravis, Neuritis, Ophthalmia, Bullous Pemphigoid, Pemphigus, Polyendocrinopathies, Purpura, Reiter's Disease, Stiff-Man Syndrome, Autoimmune Thyroiditis, Systemic Lupus Erythematosus, Autoimmune Pulmonary Inflammation, Guillain-Barre Syndrome, insulin dependent diabetes mellitis, and autoimmune inflammatory eye disease.

Similarly, allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Moreover, these molecules can be used to treat anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to treat and/or prevent organ rejection or graft-versus-host disease (GVHD). Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this case, the foreign transplanted immune cells destroy the host tissues. The administration of polynucleotides or polypeptides, or agonists or antagonists of the present invention that inhibits an immune response, particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or GVHD.

Similarly, polynucleotides or polypeptides, or agonists or antagonists of the present invention may also be used to modulate inflammation. For example, polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation and differentiation of cells involved in an inflammatory

response. These molecules can be used to treat inflammatory conditions, both chronic and acute conditions, including chronic prostatitis, granulomatous prostatitis and malacoplakia, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, or resulting from over production of cytokines (e.g., TNF or IL-1.)

Hyperproliferative Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Polynucleotides or polypeptides, or agonists or antagonists of the present invention may inhibit the proliferation of the disorder through direct or indirect interactions. Alternatively, Polynucleotides or polypeptides, or agonists or antagonists of the present invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

Examples of hyperproliferative disorders that can be treated or detected by Polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

Similarly, other hyperproliferative disorders can also be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders. paraproteinemias, purpura. sarcoidosis, Sezary Syndrome, Waldenstron's Macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

One preferred embodiment utilizes polynucleotides of the present invention to inhibit aberrant cellular division, by gene therapy using the present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the poynucleotides of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferrably an adenoviral vector (See G J. Nabel. et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention

may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use

of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other method known to one of ordinary skill in the art.

The present invention is further directed to antibody-based therapies which involve administering of anti-polypeptides and anti-polynucleotide antibodies to a mammalian, preferably human, patient for treating one or more of the described disorders. Methods for producing anti-polypeptides and anti-polynucleotide antibodies polyclonal and monoclonal antibodies are described in detail elsewhere herein. Such antibodies may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the present invention may be used therapeutically includes binding polynucleotides or polypeptides of the present invention locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of ordinary skill in the art will know how to use the antibodies of

the present invention for diagnostic. monitoring or therapeutic purposes without undue experimentation.

In particular. the antibodies. fragments and derivatives of the present invention are useful for treating a subject having or developing cell proliferative and/or differentiation disorders as described herein. Such treatment comprises administering a single or multiple doses of the antibody, or a fragment, derivative, or a conjugate thereof.

The antibodies of this invention may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors. for example., which serve to increase the number or activity of effector cells which interact with the antibodies.

It is preferred to use high affinity and/or potent in vivo inhibiting and/or neutralizing antibodies against polypeptides or polynucleotides of the present invention, fragments or regions thereof, for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragements thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides, including fragements thereof. Preferred binding affinities include those with a dissociation constant or Kd less than $5X10^{-6}M$, $10^{-6}M$, $5X10^{-7}M$, $10^{-7}M$, $5X10^{-8}M$, $10^{-8}M$, $5X10^{-9}M$, $10^{-9}M$, $5X10^{-10}M$, $10^{-10}M$, $5X10^{-11}M$, $10^{-11}M$, $5X10^{-12}M$, $10^{-12}M$, $5X10^{-13}M$, $10^{-13}M$, $5X10^{-14}M$, $10^{-14}M$, $5X10^{-15}M$, and $10^{-15}M$.

Moreover, polypeptides of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference). Antibodies directed to polypeptides or polynucleotides of the present invention may also result in inhibition of angiogenesis directly, or indirectly (See Witte L, et al.,

Cancer Metastasis Rev. 17(2):155-61 (1998), which is hereby incorporated by reference)).

Polypeptides, including protein fusions, of the present invention, or fragments thereof may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. Said polypeptides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, said polypeptides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of said proteins, either alone or in combination with small molecule drugs or adjuviants, such as apoptonin, galectins, thioredoxins, antiinflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Polypeptides, including protein fusions to, or fragments thereof, of the present invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering polypeptides, or antibodies directed to said polypeptides as described elsewere herein, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such thereapeutic affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the polypeptides of the invention (e.g., compositions containing polypeptides or polypeptide antibodes associated with heterologous

polypeptides, heterologous nucleic acids, toxins, or prodrugs) to targeted cells expressing the polypeptide of the present invention. Polypeptides or polypeptide antibodes of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. Polypeptides, protein fusions to, or fragments thereof, of the present invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the polypeptides of the present invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

Cardiovascular Disorders

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, may be used to treat cardiovascular disorders, including peripheral artery disease, such as limb ischemia.

Cardiovascular disorders include cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

Cardiovascular disorders also include heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right

ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and cardiovascular tuberculosis.

Arrhythmias include sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, and ventricular tachycardia.

Heart valve disease include aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve insufficiency, and tricuspid valve stenosis.

Myocardial diseases include alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial reperfusion injury, and myocarditis.

Myocardial ischemias include coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, angiodysplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease,

Klippel-Trenaunay-Weber Syndrome. Sturge-Weber Syndrome, angioneurotic edema, aortic diseases. Takayasu's Arteritis. aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa. cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms. thrombosis, erythromelalgia, hemorrhoids, hepatic veno-occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases. phlebitis. pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia telangiectasia, hereditary hemorrhagic telangiectasia, varicocele, varicose veins, varicose ulcer, vasculitis, and venous insufficiency.

Aneurysms include dissecting aneurysms. false aneurysms. infected aneurysms, ruptured aneurysms, aortic aneurysms, cerebral aneurysms, coronary aneurysms, heart aneurysms, and iliac aneurysms.

Arterial occlusive diseases include arteriosclerosis, intermittent claudication, carotid stenosis, fibromuscular dysplasias, mesenteric vascular occlusion, Moyamoya disease, renal artery obstruction, retinal artery occlusion, and thromboangiitis obliterans.

Cerebrovascular disorders include carotid artery diseases, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformation, cerebral artery diseases. cerebral embolism and thrombosis, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, cerebral hemorrhage, epidural hematoma, subdural hematoma, subaraxhnoid hemorrhage, cerebral infarction, cerebral ischemia (including transient), subclavian steal syndrome, periventricular leukomalacia, vascular headache, cluster headache, migraine, and vertebrobasilar insufficiency.

Embolisms include air embolisms, amniotic fluid embolisms, cholesterol embolisms, blue toe syndrome, fat embolisms, pulmonary embolisms, and thromoboembolisms. Thrombosis include coronary thrombosis, hepatic vein thrombosis, retinal vein occlusion, carotid artery thrombosis, sinus thrombosis, Wallenberg's syndrome, and thrombophlebitis.

Ischemia includes cerebral ischemia, ischemic colitis, compartment syndromes, anterior compartment syndrome, myocardial ischemia, reperfusion injuries, and peripheral limb ischemia. Vasculitis includes aortitis, arteritis, Behcet's Syndrome, Churg-Strauss Syndrome, mucocutaneous lymph node syndrome, thromboangiitis obliterans, hypersensitivity vasculitis, Schoenlein-Henoch purpura, allergic cutaneous vasculitis, and Wegener's granulomatosis.

Polynucleotides or polypeptides, or agonists or antagonists of the present invention, are especially effective for the treatment of critical limb ischemia and coronary disease.

Polypeptides may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators, gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppositorial solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Polypeptides may be administered as part of a Therapeutic, described in more detail below. Methods of delivering polynucleotides are described in more detail herein.

Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* *56*:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization

including solid tumor growth and metastases, arthritis, some types of eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, *333*:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* *29*:401-411 (1985); Folkman, Advances in Cancer Research, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Opthalmol.* *94*:715-743 (1982); and Folkman *et al.*, Science *221*:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* *235*:442-447 (1987).

The polynucleotides encoding a polypeptide of the present invention may be administered along with other polynucleotides encoding an angiogenic protein. Examples of angiogenic proteins include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of the polynucleotides and/or polypeptides of the invention, as well as agonists or antagonists of the present invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, Medicine, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)).Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist of the invention. For example, polynucleotides, polypeptides, antagonists and/or agonists

may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with polynucleotides, polypeptides, antagonists and/or agonists include, but are not limited to solid tumors, including pancreas, prostate, lung, breast, ovarian, stomach, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non- small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, polynucleotides, polypeptides, antagonists and/or agonists may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, polynucleotides, polypeptides, antagonists and/or agonists may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Polynucleotides, polypeptides, antagonists and/or agonists may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Polynucleotides, polypeptides, antagonists and/or agonists may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization;

telangiectasia; hemophiliac joints; angiofibroma: fibromuscular dysplasia: wound granulation: Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering a polynucleotide, polypeptide, antagonist and/or agonist of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention polynucleotides, polypeptides, antagonists and/or agonists are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

Moreover, Ocular disorders associated with neovascularization which can be treated with the polynucleotides and polypeptides of the present invention (including agonists and/or antagonists) include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al., Am. J. Ophthal. 85*:704-710 (1978) and Gartner *et al., Surv. Ophthal. 22*:291-312 (1978).

Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (as described above) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however,

capillaries may extend into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly

after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eyes, such that the formation of blood vessels is inhibited.

Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of a polynucleotide, polypeptide, antagonist and/or agonist to the eye, such that the formation of blood vessels is inhibited. The

compound may be administered topically, via intravitreous injection and/or via intraocular implants.

Additionally, disorders which can be treated with the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and vascular adhesions.

Moreover, disorders and/or states, which can be treated with be treated with the the polynucleotides, polypeptides, agonists and/or agonists include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uvietis, delayed wound healing, endometriosis, vascluogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis, Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (Rochele minalia quintosa), ulcers (Helicobacter pylori), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Polynucleotides, polypeptides, agonists and/or agonists may also be used in controlling menstruation or administered as either a

peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Polynucleotides, polypeptides, agonists and/or agonists of the present invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Polynucleotides, polypeptides, agonists and/or agonists may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti- angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering a polynucleotide, polypeptide, agonist and/or agonist to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly

preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, polynucleotides, polypeptides, agonists and/or agonists may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The polynucleotides, polypeptides, agonists and/or agonists of the present invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo

molybdenum complexes include molybdate. molybdenum oxide. and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates. and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, 1992); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, 1992); Cyclodextrin Tetradecasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, 1987); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, 1987); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4-chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, 1992); Thalidomide; Angostatic steroid; AGM-1470; carboxynaminolmidazole; and metalloproteinase inhibitors such as BB94.

Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as antagonists or agonists of the present invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection. In preferred embodiments, polynucleotides, polypeptides, and/or antagonists of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by polynucleotides or polypeptides, or agonists or antagonists of the present invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma,

lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated or detected by polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Wound Healing and Epithelial Cell Proliferation

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound

healing, and to stimulate hair follicle production and healing of dermal wounds. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis, eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associted with systemic treatment with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote dermal reestablishment subsequent to dermal loss

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that polynucleotides or polypeptides, agonists or antagonists of the present invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small intesting, and large intestine. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could promote proliferation of

epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Polynucleotides or polypeptides, agonists or antagonists of the present invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may have a cytoprotective effect on the small intestine mucosa. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could also be used to treat gastric and doudenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflamamatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with polynucleotides or polypeptides, agonists or antagonists of the present invention, is expected to have a significant effect on the

production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to treat diseases associate with the under expression.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to prevent and heal damage to the lungs due to various pathological states. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and brochiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of aveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary displasia, in premature infants.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetraholoride and other hepatotoxins known in the art).

In addition, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, polynucleotides or

polypeptides. as well as agonists or antagonists of the present invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

Neurological Diseases

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, polynucleotides, polypeptides, agonists and/or antagonists of the invention may be used to treat and/or detect neurologic diseases. Moreover, polynucleotides or polypeptides. or agonists or antagonists of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with polynucleotides, polypeptides, agonists, and/or antagonists of the present invention include brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema, brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis, cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease, cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and

thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache, migraine, dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, Hallervorden-Spatz Syndrome, hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, cerebral malaria, meningitis such as arachnoiditis, aseptic meningtitis such as viral meningtitis which includes lymphocytic choriomeningitis. Bacterial meningtitis which includes Haemophilus Meningtitis, Listeria Meningtitis, Meningococcal Meningtitis such as Waterhouse-Friderichsen Syndrome,

Pneumococcal Meningtitis and meningeal tuberculosis, fungal meningitis such as Cryptococcal Meningtitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie) cerebral toxoplasmosis, central nervous system neoplasms such as brain neoplasms that include cerebellear neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms, meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral sceloris which includes adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica, Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucolipidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele,

meningomyelocele. spinal dysraphism such as spina bifida cystica and spina bifida occulta. hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease. hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as Congenital Analgesia and Familial Dysautonomia. Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome. Amnesia such as retrograde amnesia, apraxia. neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia. hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia. Duane's Syndrome. Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia, respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease. muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease

and Werdnig-Hoffmann Disease. Postpoliomyelitis Syndrome. Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica. Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis, Multiplex Paramyloclonus. Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies. autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia,Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome. Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as Neuromyelitis Optica and Swayback, Diabetic neuropathies such as diabetic foot, nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculities such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica. Gustatory Sweating and Tetany).

Infectious Disease

Polynucleotides or polypeptides. as well as agonists or antagonists of the present invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may also directly inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiollitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific

embodiments, polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment polynucleotides, polypeptides, or agonists or antagonists of the invention are used to treat AIDS.

Similarly, bacterial or fungal agents that can cause disease or symptoms and that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, include, but not limited to, the following Gram-Negative and Gram-positive bacteria and bacterial families and fungi: Actinomycetales (e.g., Corynebacterium, Mycobacterium, Norcardia), Cryptococcus neoformans, Aspergillosis, Bacillaceae (e.g., Anthrax, Clostridium), Bacteroidaceae, Blastomycosis, Bordetella, Borrelia (e.g., Borrelia burgdorferi, Brucellosis, Candidiasis, Campylobacter, Coccidioidomycosis, Cryptococcosis, Dermatocycoses, E. coli (e.g., Enterotoxigenic E. coli and Enterohemorrhagic E. coli), Enterobacteriaceae (Klebsiella, Salmonella (e.g., Salmonella typhi, and Salmonella paratyphi), Serratia, Yersinia), Erysipelothrix, Helicobacter, Legionellosis, Leptospirosis, Listeria, Mycoplasmatales, Mycobacterium leprae, Vibrio cholerae, Neisseriaceae (e.g., Acinetobacter, Gonorrhea, Menigococcal), Meisseria meningitidis, Pasteurellacea Infections (e.g., Actinobacillus, Heamophilus (e.g., Heamophilus influenza type B), Pasteurella), Pseudomonas, Rickettsiaceae, Chlamydiaceae, Syphilis, Shigella spp., Staphylococcal, Meningiococcal, Pneumococcal and Streptococcal (e.g., Streptococcus pneumoniae and Group B Streptococcus). These bacterial or fungal families can cause the following diseases or symptoms, including, but not limited to: bacteremia, endocarditis, eye infections (conjunctivitis, tuberculosis, uveitis), gingivitis, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, Dysentery, Paratyphoid Fever, food poisoning,

Typhoid, pneumonia, Gonorrhea, meningitis (e.g., mengitis types A and B), Chlamydia, Syphilis, Diphtheria, Leprosy, Paratuberculosis, Tuberculosis, Lupus, Botulism, gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections. Polynucleotides or polypeptides, agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, Ppolynucleotides, polypeptides, agonists or antagonists of the invention are used to treat: tetanus, Diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated or detected by a polynucleotide or polypeptide and/or agonist or antagonist of the present invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardiasis, Helminthiasis, Leishmaniasis, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., Plasmodium virax, Plasmodium falciparium, Plasmodium malariae and Plasmodium ovale). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. polynucleotides or polypeptides, or agonists or antagonists of the invention, can be used to treat or detect any of these symptoms or diseases.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention of the present invention could either be by administering an effective amount of a polypeptide to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the polypeptide or polynucleotide of the present invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

Regeneration

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997).) The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteocarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associatedwith vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using polynucleotides or polypeptides, as well as agonists or antagonists of the present invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stoke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases

(e.g., Alzheimer's disease. Parkinson's disease. Huntington's disease. amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the polynucleotides or polypeptides, as well as agonists or antagonists of the present invention.

Chemotaxis

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation. infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection, hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract fibroblasts, which can be used to treat wounds.

It is also contemplated that polynucleotides or polypeptides, as well as agonists or antagonists of the present invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, polynucleotides or polypeptides, as well as agonists or antagonists of the present invention could be used as an inhibitor of chemotaxis.

Binding Activity

A polypeptide of the present invention may be used to screen for molecules that bind to the polypeptide or for molecules to which the polypeptide binds. The binding of the polypeptide and the molecule may activate (agonist), increase, inhibit

(antagonist), or decrease activity of the polypeptide or the molecule bound. Examples of such molecules include antibodies, oligonucleotides. proteins (e.g., receptors),or small molecules.

Preferably, the molecule is closely related to the natural ligand of the polypeptide, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991).) Similarly, the molecule can be closely related to the natural receptor to which the polypeptide binds, or at least, a fragment of the receptor capable of being bound by the polypeptide (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the polypeptide. Preferred cells include cells from mammals, yeast, Drosophila, or *E. coli*. Cells expressing the polypeptide (or cell membrane containing the expressed polypeptide) are then preferably contacted with a test compound potentially containing the molecule to observe binding, stimulation, or inhibition of activity of either the polypeptide or the molecule.

The assay may simply test binding of a candidate compound to the polypeptide, wherein binding is detected by a label, or in an assay involving competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the polypeptide.

Alternatively, the assay can be carried out using cell-free preparations, polypeptide/molecule affixed to a solid support, chemical libraries, or natural product mixtures. The assay may also simply comprise the steps of mixing a candidate compound with a solution containing a polypeptide, measuring polypeptide/molecule activity or binding, and comparing the polypeptide/molecule activity or binding to a standard.

Preferably, an ELISA assay can measure polypeptide level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure polypeptide level or activity by either binding, directly or indirectly, to the polypeptide or by competing with the polypeptide for a substrate.

Additionally, the receptor to which the polypeptide of the present invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, expression cloning is employed wherein polyadenylated RNA is prepared from a cell responsive to the polypeptides, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the polypeptides. Transfected cells which are grown on glass slides are exposed to the polypeptide of the present invention, after they have been labelled. The polypeptides can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

As an alternative approach for receptor identification, the labeled polypeptides can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule. Cross-linked material is resolved by PAGE analysis and exposed to X-ray film. The labeled complex containing the receptors of the polypeptides can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the polypeptide of the present invention thereby effectively generating agonists and antagonists of the polypeptide of the present invention. *See generally*, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., *et al.*. *Curr. Opinion Biotechnol.* 8:724-

33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., *et al., J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998) (each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides and corresponding polypeptides may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides and corresponding polypeptides may be alterred by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of the polypeptide of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal, MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the polypeptide of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of the polypeptide of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, a the polypeptide of the present invention, the compound to be screened and ^{3}[H]

thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of ^{3}[H] thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of ^{3}[H] thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for a polypeptide of the present invention is incubated with a labeled polypeptide of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential agonist or antagonist. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the polypeptide/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the polypeptides of the invention from suitably manipulated cells or tissues.

Therefore, the invention includes a method of identifying compounds which bind to a polypeptide of the invention comprising the steps of: (a) incubating a candidate binding compound with a polypeptide of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate compound with a polypeptide of the present invention, (b) assaying a biological

activity, and (b) determining if a biological activity of the polypeptide has been altered.

Targeted Delivery

In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a polypeptide of the invention, or cells expressing a cell bound form of a polypeptide of the invention.

As discussed herein, polypeptides or antibodies of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering polypeptides of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNAse, alpha

toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

Drug Screening

Further contemplated is the use of the polypeptides of the present invention, or the polynucleotides encoding these polypeptides, to screen for molecules which modify the activities of the polypeptides of the present invention. Such a method would include contacting the polypeptide of the present invention with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of these polypeptides following binding.

This invention is particularly useful for screening therapeutic compounds by using the polypeptides of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The polypeptide or fragment employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the polypeptide or fragment. Drugs are screened against such transformed cells in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and a polypeptide of the present invention.

Thus, the present invention provides methods of screening for drugs or any other agents which affect activities mediated by the polypeptides of the present invention. These methods comprise contacting such an agent with a polypeptide of the present invention or a fragment thereof and assaying for the presence of a

complex between the agent and the polypeptide or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the polypeptides of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the polypeptides of the present invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with polypeptides of the present invention and washed. Bound polypeptides are then detected by methods well known in the art. Purified polypeptides are coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding polypeptides of the present invention specifically compete with a test compound for binding to the polypeptides or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with a polypeptide of the invention.

Antisense And Ribozyme (Antagonists)

In specific embodiments, antagonists according to the present invention are nucleic acids corresponding to the sequences contained in SEQ ID NO:X, or the complementary strand thereof, and/or to nucleotide sequences contained in the cDNA contained in the related cDNA clone identified in Table 1. In one embodiment, antisense sequence is generated internally, by the organism, in another embodiment, the antisense sequence is separately administered (see, for example, O'Connor, J.,

Neurochem. 56:560 (1991). Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Antisense technology can be used to control gene expression through antisense DNA or RNA, or through triple-helix formation. Antisense techniques are discussed for example, in Okano, J., Neurochem. 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988). Triple helix formation is discussed in, for instance, Lee et al., Nucleic Acids Research 6:3073 (1979); Cooney et al., Science 241:456 (1988); and Dervan et al., Science 251:1300 (1991). The methods are based on binding of a polynucleotide to a complementary DNA or RNA.

For example, the use of c-myc and c-myb antisense RNA constructs to inhibit the growth of the non-lymphocytic leukemia cell line HL-60 and other cell lines was previously described. (Wickstrom et al. (1988); Anfossi et al. (1989)). These experiments were performed in vitro by incubating cells with the oligoribonucleotide. A similar procedure for in vivo use is described in WO 91/15580. Briefly, a pair of oligonucleotides for a given antisense RNA is produced as follows: A sequence complimentary to the first 15 bases of the open reading frame is flanked by an EcoR1 site on the 5 end and a HindIII site on the 3 end. Next, the pair of oligonucleotides is heated at 90°C for one minute and then annealed in 2X ligation buffer (20mM TRIS HCl pH 7.5, 10mM MgCl2, 10MM dithiothreitol (DTT) and 0.2 mM ATP) and then ligated to the EcoR1/Hind III site of the retroviral vector PMV7 (WO 91/15580).

For example, the 5' coding portion of a polynucleotide that encodes the polypeptide of the present invention may be used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription thereby preventing transcription and the production of the receptor. The antisense RNA oligonucleotide hybridizes to the mRNA in vivo and blocks translation of the mRNA molecule into receptor polypeptide.

In one embodiment, the antisense nucleic acid of the invention is produced intracellularly by transcription from an exogenous sequence. For example, a vector or a portion thereof, is transcribed, producing an antisense nucleic acid (RNA) of the

invention. Such a vector would contain a sequence encoding the antisense nucleic acid. Such a vector can remain episomal or become chromosomally integrated, as long as it can be transcribed to produce the desired antisense RNA. Such vectors can be constructed by recombinant DNA technology methods standard in the art. Vectors can be plasmid, viral, or others known in the art, used for replication and expression in vertebrate cells. Expression of the sequence encoding the polypeptide of the present invnetion or fragments thereof, can be by any promoter known in the art to act in vertebrate, preferably human cells. Such promoters can be inducible or constitutive. Such promoters include, but are not limited to, the SV40 early promoter region (Bernoist and Chambon, Nature 29:304-310 (1981), the promoter contained in the 3' long terminal repeat of Rous sarcoma virus (Yamamoto et al., Cell 22:787-797 (1980), the herpes thymidine promoter (Wagner et al., Proc. Natl. Acad. Sci. U.S.A. 78:1441-1445 (1981), the regulatory sequences of the metallothionein gene (Brinster, et al., Nature 296:39-42 (1982)), etc.

The antisense nucleic acids of the invention comprise a sequence complementary to at least a portion of an RNA transcript of a gene of the present invention. However, absolute complementarity, although preferred, is not required. A sequence "complementary to at least a portion of an RNA," referred to herein, means a sequence having sufficient complementarity to be able to hybridize with the RNA, forming a stable duplex; in the case of double stranded antisense nucleic acids, a single strand of the duplex DNA may thus be tested, or triplex formation may be assayed. The ability to hybridize will depend on both the degree of complementarity and the length of the antisense nucleic acid. Generally, the larger the hybridizing nucleic acid, the more base mismatches with a RNA it may contain and still form a stable duplex (or triplex as the case may be). One skilled in the art can ascertain a tolerable degree of mismatch by use of standard procedures to determine the melting point of the hybridized complex.

Oligonucleotides that are complementary to the 5' end of the message, e.g., the 5' untranslated sequence up to and including the AUG initiation codon, should work most efficiently at inhibiting translation. However, sequences complementary to the

3' untranslated sequences of mRNAs have been shown to be effective at inhibiting translation of mRNAs as well. See generally, Wagner, R., 1994, Nature 372:333-335. Thus, oligonucleotides complementary to either the 5'- or 3'- non- translated, non-coding regions of polynucleotide sequences described herein could be used in an antisense approach to inhibit translation of endogenous mRNA. Oligonucleotides complementary to the 5' untranslated region of the mRNA should include the complement of the AUG start codon. Antisense oligonucleotides complementary to mRNA coding regions are less efficient inhibitors of translation but could be used in accordance with the invention. Whether designed to hybridize to the 5'-, 3'- or coding region of mRNA of the present invention, antisense nucleic acids should be at least six nucleotides in length, and are preferably oligonucleotides ranging from 6 to about 50 nucleotides in length. In specific aspects the oligonucleotide is at least 10 nucleotides, at least 17 nucleotides, at least 25 nucleotides or at least 50 nucleotides.

The polynucleotides of the invention can be DNA or RNA or chimeric mixtures or derivatives or modified versions thereof, single-stranded or double-stranded. The oligonucleotide can be modified at the base moiety, sugar moiety, or phosphate backbone, for example, to improve stability of the molecule, hybridization, etc. The oligonucleotide may include other appended groups such as peptides (e.g., for targeting host cell receptors in vivo), or agents facilitating transport across the cell membrane (see, e.g., Letsinger et al., 1989, Proc. Natl. Acad. Sci. U.S.A. 86:6553-6556; Lemaitre et al., 1987, Proc. Natl. Acad. Sci. 84:648-652; PCT Publication No. WO88/09810, published December 15, 1988) or the blood-brain barrier (see, e.g., PCT Publication No. WO89/10134, published April 25, 1988), hybridization-triggered cleavage agents. (See, e.g., Krol et al., 1988, BioTechniques 6:958-976) or intercalating agents. (See, e.g., Zon, 1988, Pharm. Res. 5:539-549). To this end, the oligonucleotide may be conjugated to another molecule, e.g., a peptide, hybridization triggered cross-linking agent, transport agent, hybridization-triggered cleavage agent, etc.

The antisense oligonucleotide may comprise at least one modified base moiety which is selected from the group including, but not limited to, 5-fluorouracil,

5-bromouracil, 5-chlorouracil. 5-iodouracil. hypoxanthine, xantine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil. 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine.

The antisense oligonucleotide may also comprise at least one modified sugar moiety selected from the group including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose.

In yet another embodiment, the antisense oligonucleotide comprises at least one modified phosphate backbone selected from the group including, but not limited to, a phosphorothioate, a phosphorodithioate, a phosphoramidothioate, a phosphoramidate, a phosphordiamidate. a methylphosphonate, an alkyl phosphotriester, and a formacetal or analog thereof.

In yet another embodiment, the antisense oligonucleotide is an a-anomeric oligonucleotide. An a-anomeric oligonucleotide forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual b-units, the strands run parallel to each other (Gautier et al., 1987, Nucl. Acids Res. 15:6625-6641). The oligonucleotide is a 2'-0-methylribonucleotide (Inoue et al., 1987, Nucl. Acids Res. 15:6131-6148), or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

Polynucleotides of the invention may be synthesized by standard methods known in the art. e.g. by use of an automated DNA synthesizer (such as are

commercially available from Biosearch, Applied Biosystems, etc.). As examples, phosphorothioate oligonucleotides may be synthesized by the method of Stein et al. (1988, Nucl. Acids Res. 16:3209), methylphosphonate oligonucleotides can be prepared by use of controlled pore glass polymer supports (Sarin et al., 1988, Proc. Natl. Acad. Sci. U.S.A. 85:7448-7451), etc.

While antisense nucleotides complementary to the coding region sequence could be used, those complementary to the transcribed untranslated region are most preferred.

Potential antagonists according to the invention also include catalytic RNA, or a ribozyme (See, e.g., PCT International Publication WO 90/11364, published October 4, 1990; Sarver et al, Science 247:1222-1225 (1990). While ribozymes that cleave mRNA at site specific recognition sequences can be used to destroy mRNAs, the use of hammerhead ribozymes is preferred. Hammerhead ribozymes cleave mRNAs at locations dictated by flanking regions that form complementary base pairs with the target mRNA. The sole requirement is that the target mRNA have the following sequence of two bases: 5'-UG-3'. The construction and production of hammerhead ribozymes is well known in the art and is described more fully in Haseloff and Gerlach, Nature 334:585-591 (1988). There are numerous potential hammerhead ribozyme cleavage sites within the nucleotide sequence of SEQ ID NO:X. Preferably, the ribozyme is engineered so that the cleavage recognition site is located near the 5' end of the mRNA: i.e., to increase efficiency and minimize the intracellular accumulation of non-functional mRNA transcripts.

As in the antisense approach, the ribozymes of the invention can be composed of modified oligonucleotides (e.g. for improved stability, targeting, etc.) and should be delivered to cells which express in vivo. DNA constructs encoding the ribozyme may be introduced into the cell in the same manner as described above for the introduction of antisense encoding DNA. A preferred method of delivery involves using a DNA construct "encoding" the ribozyme under the control of a strong constitutive promoter, such as, for example, pol III or pol II promoter, so that transfected cells will produce sufficient quantities of the ribozyme to destroy

endogenous messages and inhibit translation. Since ribozymes unlike antisense molecules, are catalytic, a lower intracellular concentration is required for efficiency.

Antagonist/agonist compounds may be employed to inhibit the cell growth and proliferation effects of the polypeptides of the present invention on neoplastic cells and tissues, i.e. stimulation of angiogenesis of tumors, and, therefore, retard or prevent abnormal cellular growth and proliferation, for example, in tumor formation or growth.

The antagonist/agonist may also be employed to prevent hyper-vascular diseases, and prevent the proliferation of epithelial lens cells after extracapsular cataract surgery. Prevention of the mitogenic activity of the polypeptides of the present invention may also be desirous in cases such as restenosis after balloon angioplasty.

The antagonist/agonist may also be employed to prevent the growth of scar tissue during wound healing.

The antagonist/agonist may also be employed to treat the diseases described herein.

Thus, the invention provides a method of treating disorders or diseases, including but not limited to the disorders or diseases listed throughout this application, associated with overexpression of a polynucleotide of the present invention by administering to a patient (a) an antisense molecule directed to the polynucleotide of the present invention, and/or (b) a ribozyme directed to the polynucleotide of the present invention.

Other Activities

A polypeptide, polynucleotide, agonist, or antagonist of the present invention, as a result of the ability to stimulate vascular endothelial cell growth, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The polypeptide, polynucleotide, agonist, or antagonist of

the present invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may be also be employed to prevent skin aging due to sunburn by stimulating keratinocyte growth.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth. Along the same lines, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

A polypeptide. polynucleotide. agonist. or antagonist of the present invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, a polypeptide, polynucleotide, agonist, or antagonist of the present invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

A polypeptide, polynucleotide. agonist, or antagonist of the present invention may be used to change a mammal's mental state or physical state by influencing biorhythms, caricadic rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, libido, memory, stress, or other cognitive qualities.

A polypeptide, polynucleotide, agonist, or antagonist of the present invention may also be used as a food additive or preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate, and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

<u>Other Preferred Embodiments</u>

Other preferred embodiments of the claimed invention include an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 50 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is a nucleic acid molecule wherein said sequence of contiguous nucleotides is included in the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 150 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least about 500 contiguous nucleotides in the nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the nucleotide sequence of SEQ ID NO:X in the range of positions identified as "Start" and "End" in columns 7 and 8 as defined for SEQ ID NO:X in Table 1.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule which hybridizes under stringent hybridization conditions to a nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto, and/or the cDNA in

the related cDNA clone contained in the deposit. wherein said nucleic acid molecule which hybridizes does not hybridize under stringent hybridization conditions to a nucleic acid molecule having a nucleotide sequence consisting of only A residues or of only T residues.

Also preferred is a composition of matter comprising a DNA molecule which comprises a cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in the nucleotide sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule, wherein said sequence of at least 50 contiguous nucleotides is included in the nucleotide sequence of an open reading frame sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 150 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to sequence of at least 500 contiguous nucleotides in the nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to the complete nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

A further preferred embodiment is a method for detecting in a biological sample a nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the

complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit; which method comprises a step of comparing a nucleotide sequence of at least one nucleic acid molecule in said sample with a sequence selected from said group and determining whether the sequence of said nucleic acid molecule in said sample is at least 95% identical to said selected sequence.

Also preferred is the above method wherein said step of comparing sequences comprises determining the extent of nucleic acid hybridization between nucleic acid molecules in said sample and a nucleic acid molecule comprising said sequence selected from said group. Similarly, also preferred is the above method wherein said step of comparing sequences is performed by comparing the nucleotide sequence determined from a nucleic acid molecule in said sample with said sequence selected from said group. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

A further preferred embodiment is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting nucleic acid molecules in said sample, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleotide sequence of SEQ ID NO:X; or the cDNA in the related cDNA clone identified in Table 1 which encodes a protein, wherein the method comprises a step of detecting in a biological sample

obtained from said subject nucleic acid molecules, if any, comprising a nucleotide sequence that is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto: and a nucleotide sequence of the cDNA in the related cDNA clone contained in the deposit.

Also preferred is the above method for diagnosing a pathological condition which comprises a step of detecting nucleic acid molecules comprising a nucleotide sequence in a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from said group.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a panel of at least two nucleotide sequences, wherein at least one sequence in said panel is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the related cDNA clone contained in the deposit. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is a composition of matter comprising isolated nucleic acid molecules wherein the nucleotide sequences of said nucleic acid molecules comprise a DNA microarray or "chip" of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 100, 150, 200, 250, 300, 500, 1000, 2000, 3000 or 4000 nucleotide sequences, wherein at least one sequence in said DNA microarray or "chip" is at least 95% identical to a sequence of at least 50 contiguous nucleotides in a sequence selected from the group consisting of: a nucleotide sequence of SEQ ID NO:X or the complementary strand thereto; and a nucleotide sequence encoded by the cDNA in the cDNA clone referenced in Table 1. The nucleic acid molecules can comprise DNA molecules or RNA molecules.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the

polypeptide sequence of SEQ ID NO:Y: a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the complete amino acid sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and/or a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is an isolated polypeptide comprising an amino acid sequence at least 90% identical to a sequence of at least about 10 contiguous amino acids in the complete amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is a polypeptide wherein said sequence of contiguous amino acids is included in the amino acid sequence of a portion of said polypeptide encoded by the cDNA clone referenced in Table 1; a polypeptide encoded by SEQ ID NO:X; and/or the polypeptide sequence of SEQ ID NO:Y.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 30 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence of at least about 100 contiguous amino acids in the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Also preferred is an isolated polypeptide comprising an amino acid sequence at least 95% identical to the amino acid sequence of a polypeptide encoded by the cDNA clone referenced in Table 1.

Further preferred is an isolated antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone contained in the deposit.

Further preferred is a method for detecting in a biological sample a polypeptide comprising an amino acid sequence which is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1; which method comprises a step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group and determining whether the sequence of said polypeptide molecule in said sample is at least 90% identical to said sequence of at least 10 contiguous amino acids.

Also preferred is the above method wherein said step of comparing an amino acid sequence of at least one polypeptide molecule in said sample with a sequence selected from said group comprises determining the extent of specific binding of polypeptides in said sample to an antibody which binds specifically to a polypeptide comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: a polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X;

and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method wherein said step of comparing sequences is performed by comparing the amino acid sequence determined from a polypeptide molecule in said sample with said sequence selected from said group.

Also preferred is a method for identifying the species, tissue or cell type of a biological sample which method comprises a step of detecting polypeptide molecules in said sample, if any, comprising an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is the above method for identifying the species, tissue or cell type of a biological sample, which method comprises a step of detecting polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the above group.

Also preferred is a method for diagnosing in a subject a pathological condition associated with abnormal structure or expression of a nucleic acid sequence identified in Table 1 encoding a polypeptide, which method comprises a step of detecting in a biological sample obtained from said subject polypeptide molecules comprising an amino acid sequence in a panel of at least two amino acid sequences, wherein at least one sequence in said panel is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

In any of these methods, the step of detecting said polypeptide molecules includes using an antibody.

Also preferred is an isolated nucleic acid molecule comprising a nucleotide sequence which is at least 95% identical to a nucleotide sequence encoding a polypeptide wherein said polypeptide comprises an amino acid sequence that is at least 90% identical to a sequence of at least 10 contiguous amino acids in a sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X: and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Also preferred is an isolated nucleic acid molecule, wherein said nucleotide sequence encoding a polypeptide has been optimized for expression of said polypeptide in a prokaryotic host.

Also preferred is an isolated nucleic acid molecule, wherein said polypeptide comprises an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1.

Further preferred is a method of making a recombinant vector comprising inserting any of the above isolated nucleic acid molecule into a vector. Also preferred is the recombinant vector produced by this method. Also preferred is a method of making a recombinant host cell comprising introducing the vector into a host cell, as well as the recombinant host cell produced by this method.

Also preferred is a method of making an isolated polypeptide comprising culturing this recombinant host cell under conditions such that said polypeptide is expressed and recovering said polypeptide. Also preferred is this method of making an isolated polypeptide, wherein said recombinant host cell is a eukaryotic cell and said polypeptide is a human protein comprising an amino acid sequence selected from the group consisting of: polypeptide sequence of SEQ ID NO:Y; a polypeptide encoded by SEQ ID NO:X; and a polypeptide encoded by the cDNA in the related cDNA clone referenced in Table 1. The isolated polypeptide produced by this method is also preferred.

Also preferred is a method of treatment of an individual in need of an increased level of a protein activity, which method comprises administering to such

an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide. immunogenic fragment or analogue thereof, binding agent. antibody, or antigen binding fragment of the claimed invention effective to increase the level of said protein activity in said individual.

Also preferred is a method of treatment of an individual in need of a decreased level of a protein activity, which method comprised administering to such an individual a Therapeutic comprising an amount of an isolated polypeptide, polynucleotide, immunogenic fragment or analogue thereof, binding agent, antibody, or antigen binding fragment of the claimed invention effective to decrease the level of said protein activity in said individual.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Examples

Example 1: Isolation of a Selected cDNA Clone From the Deposited Sample

Each deposited cDNA clone is contained in a plasmid vector. Table 5 identifies the vectors used to construct the cDNA library from which each clone was isolated. In many cases, the vector used to construct the library is a phage vector from which a plasmid has been excised. The following correlates the related plasmid for each phage vector used in constructing the cDNA library. For example, where a particular clone is identified in Table 5 as being isolated in the vector "Lambda Zap," the corresponding deposited clone is in "pBluescript."

Vector Used to Construct Library	Corresponding Deposited Plasmid
Lambda Zap	pBluescript (pBS)
Uni-Zap XR	pBluescript (pBS)
Zap Express	pBK
lafmid BA	plafmid BA
pSport1	pSport1
pCMVSport 2.0	pCMVSport 2.0
pCMVSport 3.0	pCMVSport 3.0
pCR®2.1	pCR®2.1

Vectors Lambda Zap (U.S. Patent Nos. 5,128.256 and 5,286,636), Uni-Zap XR (U.S. Patent Nos. 5,128, 256 and 5,286,636), Zap Express (U.S. Patent Nos. 5,128,256 and 5,286,636), pBluescript (pBS) (Short, J. M. et al., Nucleic Acids Res. 16:7583-7600 (1988); Alting-Mees, M. A. and Short, J. M., Nucleic Acids Res. 17:9494 (1989)) and pBK (Alting-Mees, M. A. et al., Strategies 5:58-61 (1992)) are commercially available from Stratagene Cloning Systems, Inc., 11011 N. Torrey Pines Road, La Jolla, CA, 92037. pBS contains an ampicillin resistance gene and pBK contains a neomycin resistance gene. Both can be transformed into E. coli strain XL-1 Blue, also available from Stratagene. pBS comes in 4 forms SK+, SK-, KS+ and KS. The S and K refers to the orientation of the polylinker to the T7 and T3

primer sequences which flank the polylinker region ("S" is for SacI and "K" is for KpnI which are the first sites on each respective end of the linker). "+" or "-" refer to the orientation of the f1 origin of replication ("ori"), such that in one orientation, single stranded rescue initiated from the f1 ori generates sense strand DNA and in the other, antisense.

Vectors pSport1, pCMVSport 2.0 and pCMVSport 3.0, were obtained from Life Technologies, Inc., P. O. Box 6009, Gaithersburg, MD 20897. All Sport vectors contain an ampicillin resistance gene and may be transformed into E. coli strain DH10B, also available from Life Technologies. (See, for instance, Gruber, C. E., et al., Focus 15:59 (1993).) Vector lafmid BA (Bento Soares, Columbia University, NY) contains an ampicillin resistance gene and can be transformed into E. coli strain XL-1 Blue. Vector pCR®2.1, which is available from Invitrogen, 1600 Faraday Avenue, Carlsbad, CA 92008, contains an ampicillin resistance gene and may be transformed into E. coli strain DH10B, available from Life Technologies. (See, for instance, Clark, J. M., Nuc. Acids Res. 16:9677-9686 (1988) and Mead, D. et al., Bio/Technology 9: (1991).) Preferably, a polynucleotide of the present invention does not comprise the phage vector sequences identified for the particular clone in Table 5, as well as the corresponding plasmid vector sequences designated above.

The deposited material in the sample assigned the ATCC Deposit Number cited by reference to Table 2 and 5 for any given cDNA clone also may contain one or more additional plasmids, each comprising a cDNA clone different from that given clone. Thus, deposits sharing the same ATCC Deposit Number contain at least a plasmid for each cDNA clone referenced in Table 1.

TABLE 5

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HUKA HUKB HUKC HUKD HUKE HUKF HUKG	Human Uterine Cancer	Lambda ZAP II	LP01
HCNA HCNB	Human Colon	Lambda Zap II	LP01
HFFA	Human Fetal Brain, random primed	Lambda Zap II	LP01
HTWA	Resting T-Cell	Lambda ZAP II	LP01
HBQA	Early Stage Human Brain, random primed	Lambda ZAP II	LP01
HLMB HLMF HLMG HLMH HLMI HLMJ HLMM HLMN	breast lymph node CDNA library	Lambda ZAP II	LP01
HCQA HCQB	human colon cancer	Lamda ZAP II	LP01
HMEA HMEC HMED HMEE HMEF HMEG HMEI HMEJ HMEK HMEL	Human Microvascular Endothelial Cells, fract. A	Lambda ZAP II	LP01
HUSA HUSC	Human Umbilical Vein Endothelial Cells, fract. A	Lambda ZAP II	LP01
HLQA HLQB	Hepatocellular Tumor	Lambda ZAP II	LP01
HHGA HHGB HHGC HHGD	Hemangiopericytoma	Lambda ZAP II	LP01
HSDM	Human Striatum Depression, re-rescue	Lambda ZAP II	LP01
HUSH	H Umbilical Vein Endothelial Cells, frac A, re-excision	Lambda ZAP II	LP01
HSGS	Salivary gland, subtracted	Lambda ZAP II	LP01
HFXA HFXB HFXC HFXD HFXE HFXF HFXG HFXH	Brain frontal cortex	Lambda ZAP II	LP01
HPQA HPQB HPQC	PERM TF274	Lambda ZAP II	LP01
HFXJ HFXK	Brain Frontal Cortex, re-excision	Lambda ZAP II	LP01
HCWA HCWB HCWC HCWD HCWE HCWF HCWG HCWH HCWI HCWJ HCWK	CD34 positive cells (Cord Blood)	ZAP Express	LP02
HCUA HCUB HCUC	CD34 depleted Buffy Coat (Cord Blood)	ZAP Express	LP02
HRSM	A-14 cell line	ZAP Express	LP02
HRSA	A1-CELL LINE	ZAP Express	LP02
HCUD HCUE HCUF HCUG HCUH HCUI	CD34 depleted Buffy Coat (Cord Blood), re-excision	ZAP Express	LP02
HBXE HBXF HBXG	H. Whole Brain #2, re-excision	ZAP Express	LP02
HRLM	L8 cell line	ZAP Express	LP02
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP02
HUDA HUDB HUDC	Testes	ZAP Express	LP02
HHTM HHTN HHTO	H. hypothalamus, frac A;re-excision	ZAP Express	LP02
HHTL	H. hypothalamus, frac A	ZAP Express	LP02
HASA HASD	Human Adult Spleen	Uni-ZAP XR	LP03
HFKC HFKD HFKE HFKF HFKG	Human Fetal Kidney	Uni-ZAP XR	LP03
HE8A HE8B HE8C HE8D HE8E HE8F HE8M HE8N	Human 8 Week Whole Embryo	Uni-ZAP XR	LP03
HGBA HGBD HGBE HGBF HGBG HGBH HGBI	Human Gall Bladder	Uni-ZAP XR	LP03
HLHA HLHB HLHC HLHD HLHE	Human Fetal Lung III	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLHF HLHG HLHH HLHQ			
HPMA HPMB HPMC HPMD HPME HPMF HPMG HPMH	Human Placenta	Uni-ZAP XR	LP03
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP03
HSIA HSIC HSID HSIE	Human Adult Small Intestine	Uni-ZAP XR	LP03
HTEA HTEB HTEC HTED HTEE HTEF HTEG HTEH HTEI HTEJ HTEK	Human Testes	Uni-ZAP XR	LP03
HTPA HTPB HTPC HTPD HTPE	Human Pancreas Tumor	Uni-ZAP XR	LP03
HTTA HTTB HTTC HTTD HTTE HTTF	Human Testes Tumor	Uni-ZAP XR	LP03
HAPA HAPB HAPC HAPM	Human Adult Pulmonary	Uni-ZAP XR	LP03
HETA HETB HETC HETD HETE HETF HETG HETH HETI	Human Endometrial Tumor	Uni-ZAP XR	LP03
HHFB HHFC HHFD HHFE HHFF HHFG HHFH HHFI	Human Fetal Heart	Uni-ZAP XR	LP03
HHPB HHPC HHPD HHPE HHPF HHPG HHPH	Human Hippocampus	Uni-ZAP XR	LP03
HCE1 HCE2 HCE3 HCE4 HCE5 HCEB HCEC HCED HCEE HCEF HCEG	Human Cerebellum	Uni-ZAP XR	LP03
HUVB HUVC HUVD HUVE	Human Umbilical Vein. Endo. remake	Uni-ZAP XR	LP03
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP03
HTAA HTAB HTAC HTAD HTAE	Human Activated T-Cells	Uni-ZAP XR	LP03
HFEA HFEB HFEC	Human Fetal Epithelium (Skin)	Uni-ZAP XR	LP03
HJPA HJPB HJPC HJPD	HUMAN JURKAT MEMBRANE BOUND POLYSOMES	Uni-ZAP XR	LP03
HESA	Human epithelioid sarcoma	Uni-Zap XR	LP03
HLTA HLTB HLTC HLTD HLTE HLTF	Human T-Cell Lymphoma	Uni-ZAP XR	LP03
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP03
HRDA HRDB HRDC HRDD HRDE HRDF	Human Rhabdomyosarcoma	Uni-ZAP XR	LP03
HCAA HCAB HCAC	Cem cells cyclohexamide treated	Uni-ZAP XR	LP03
HRGA HRGB HRGC HRGD	Raji Cells. cyclohexamide treated	Uni-ZAP XR	LP03
HSUA HSUB HSUC HSUM	Supt Cells. cyclohexamide treated	Uni-ZAP XR	LP03
HT4A HT4C HT4D	Activated T-Cells. 12 hrs.	Uni-ZAP XR	LP03
HE9A HE9B HE9C HE9D HE9E HE9F HE9G HE9H HE9M HE9N	Nine Week Old Early Stage Human	Uni-ZAP XR	LP03
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP03
HT5A	Activated T-Cells. 24 hrs.	Uni-ZAP XR	LP03
HFGA HFGM	Human Fetal Brain	Uni-ZAP XR	LP03
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP03
HBGB HBGD	Human Primary Breast Cancer	Uni-ZAP XR	LP03
HBNA HBNB	Human Normal Breast	Uni-ZAP XR	LP03
HCAS	Cem Cells, cyclohexamide treated. subtra	Uni-ZAP XR	LP03
HHPS	Human Hippocampus. subtracted	pBS	LP03
HKCS HKCU	Human Colon Cancer. subtracted	pBS	LP03
HRGS	Raji cells. cyclohexamide treated. subtracted	pBS	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSUT	Supt cells. cyclohexamide treated. differentially expressed	pBS	LP03
HT4S	Activated T-Cells. 12 hrs. subtracted	Uni-ZAP XR	LP03
HCDA HCDB HCDC HCDD HCDE	Human Chondrosarcoma	Uni-ZAP XR	LP03
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP03
HTLA HTLB HTLC HTLD HTLE HTLF	Human adult testis. large inserts	Uni-ZAP XR	LP03
HLMA HLMC HLMD	Breast Lymph node cDNA library	Uni-ZAP XR	LP03
H6EA H6EB H6EC	HL-60. PMA 4H	Uni-ZAP XR	LP03
HTXA HTXB HTXC HTXD HTXE HTXF HTXG HTXH	Activated T-Cell (12hs)/Thiouridine labelledEco	Uni-ZAP XR	LP03
HNFA HNFB HNFC HNFD HNFE HNFF HNFG HNFH HNFJ	Human Neutrophil. Activated	Uni-ZAP XR	LP03
HTOB HTOC	HUMAN TONSILS. FRACTION 2	Uni-ZAP XR	LP03
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP03
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP03
HORB	Human OB HOS treated (10 nM E2) fraction I	Uni-ZAP XR	LP03
HSVA HSVB HSVC	Human Chronic Synovitis	Uni-ZAP XR	LP03
HROA	HUMAN STOMACH	Uni-ZAP XR	LP03
HBJA HBJB HBJC HBJD HBJE HBJF HBJG HBJH HBJI HBJJ HBJK	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP03
HCRA HCRB HCRC	human corpus colosum	Uni-ZAP XR	LP03
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP03
HDSA	Dermatofibrosarcoma Protuberance	Uni-ZAP XR	LP03
HMWA HMWB HMWC HMWD HMWE HMWF HMWG HMWH HMWI HMWJ	Bone Marrow Cell Line (RS4;11)	Uni-ZAP XR	LP03
HSOA	stomach cancer (human)	Uni-ZAP XR	LP03
HERA	SKIN	Uni-ZAP XR	LP03
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP03
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP03
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP03
HBCA HBCB	H. Lymph node breast Cancer	Uni-ZAP XR	LP03
HPWT	Human Prostate BPH, re-excision	Uni-ZAP XR	LP03
HFVG HFVH HFVI	Fetal Liver. subtraction II	pBS	LP03
HNFI	Human Neutrophils. Activated. re-excision	pBS	LP03
HBMB HBMC HBMD	Human Bone Marrow. re-excision	pBS	LP03
HKML HKMM HKMN	H. Kidney Medulla. re-excision	pBS	LP03
HKIX HKIY	H. Kidney Cortex. subtracted	pBS	LP03
HADT	H. Amygdala Depression. subtracted	pBS	LP03
H6AS	Hl-60. untreated. subtracted	Uni-ZAP XR	LP03
H6ES	HL-60. PMA 4H, subtracted	Uni-ZAP XR	LP03
H6BS	HL-60. RA 4h. Subtracted	Uni-ZAP XR	LP03
H6CS	HL-60. PMA 1d. subtracted	Uni-ZAP XR	LP03
HTXJ HTXK	Activated T-cell(12h)/Thiouridine-re-	Uni-ZAP XR	LP03

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
	excision		
HMSA HMSB HMSC HMSD HMSE HMSF HMSG HMSH HMSI HMSJ HMSK	Monocyte activated	Uni-ZAP XR	LP03
HAGA HAGB HAGC HAGD HAGE HAGF	Human Amygdala	Uni-ZAP XR	LP03
HSRA HSRB HSRE	STROMAL -OSTEOCLASTOMA	Uni-ZAP XR	LP03
HSRD HSRF HSRG HSRH	Human Osteoclastoma Stromal Cells - unamplified	Uni-ZAP XR	LP03
HSQA HSQB HSQC HSQD HSQE HSQF HSQG	Stromal cell TF274	Uni-ZAP XR	LP03
HSKA HSKB HSKC HSKD HSKE HSKF HSKZ	Smooth muscle, serum treated	Uni-ZAP XR	LP03
HSLA HSLB HSLC HSLD HSLE HSLF HSLG	Smooth muscle.control	Uni-ZAP XR	LP03
HSDA HSDD HSDE HSDF HSDG HSDH	Spinal cord	Uni-ZAP XR	LP03
HPWS	Prostate-BPH subtracted II	pBS	LP03
HSKW HSKX HSKY	Smooth Muscle- HASTE normalized	pBS	LP03
HFPB HFPC HFPD	H. Frontal cortex.epileptic:re-excision	Uni-ZAP XR	LP03
HSDI HSDJ HSDK	Spinal Cord. re-excision	Uni-ZAP XR	LP03
HSKN HSKO	Smooth Muscle Serum Treated. Norm	pBS	LP03
HSKG HSKH HSKI	Smooth muscle. serum induced.re-exc	pBS	LP03
HFCA HFCB HFCC HFCD HFCE HFCF	Human Fetal Brain	Uni-ZAP XR	LP04
HPTA HPTB HPTD	Human Pituitary	Uni-ZAP XR	LP04
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP04
HE6B HE6C HE6D HE6E HE6F HE6G HE6S	Human Whole Six Week Old Embryo	Uni-ZAP XR	LP04
HSSA HSSB HSSC HSSD HSSE HSSF HSSG HSSH HSSI HSSJ HSSK	Human Synovial Sarcoma	Uni-ZAP XR	LP04
HE7T	7 Week Old Early Stage Human. subtracted	Uni-ZAP XR	LP04
HEPA HEPB HEPC	Human Epididymus	Uni-ZAP XR	LP04
HSNA HSNB HSNC HSNM HSNN	Human Synovium	Uni-ZAP XR	LP04
HPFB HPFC HPFD HPFE	Human Prostate Cancer. Stage C fraction	Uni-ZAP XR	LP04
HE2A HE2D HE2E HE2H HE2I HE2M HE2N HE2O	12 Week Old Early Stage Human	Uni-ZAP XR	LP04
HE2B HE2C HE2F HE2G HE2P HE2Q	12 Week Old Early Stage Human. II	Uni-ZAP XR	LP04
HPTS HPTT HPTU	Human Pituitary. subtracted	Uni-ZAP XR	LP04
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP04
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP04
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP04
HBSD	Bone Cancer. re-excision	Uni-ZAP XR	LP04
HSGB	Salivary gland. re-excision	Uni-ZAP XR	LP04
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP04
HSXA HSXB HSXC HSXD	Human Substantia Nigra	Uni-ZAP XR	LP04
HSHA HSHB HSHC	Smooth muscle. IL1b induced	Uni-ZAP XR	LP04
HOUA HOUB HOUC HOUD HOUE	Adipocytes	Uni-ZAP XR	LP04

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP04
HELA HELB HELC HELD HELE HELF HELG HELH	Endothelial cells-control	Uni-ZAP XR	LP04
HEMA HEMB HEMC HEMD HEME HEMF HEMG HEMH	Endothelial-induced	Uni-ZAP XR	LP04
HBIA HBIB HBIC	Human Brain, Striatum	Uni-ZAP XR	LP04
HHSA HHSB HHSC HHSD HHSE	Human Hypothalmus.Schizophrenia	Uni-ZAP XR	LP04
HNGA HNGB HNGC HNGD HNGE HNGF HNGG HNGH HNGI HNGJ	neutrophils control	Uni-ZAP XR	LP04
HNHA HNHB HNHC HNHD HNHE HNHF HNHG HNHH HNHI HNHJ	Neutrophils IL-1 and LPS induced	Uni-ZAP XR	LP04
HSDB HSDC	STRIATUM DEPRESSION	Uni-ZAP XR	LP04
HHPT	Hypothalamus	Uni-ZAP XR	LP04
HSAT HSAU HSAV HSAW HSAX HSAY HSAZ	Anergic T-cell	Uni-ZAP XR	LP04
HBMS HBMT HBMU HBMV HBMW HBMX	Bone marrow	Uni-ZAP XR	LP04
HOEA HOEB HOEC HOED HOEE HOEF HOEJ	Osteoblasts	Uni-ZAP XR	LP04
HAIA HAIB HAIC HAID HAIE HAIF	Epithelial-TNFa and INF induced	Uni-ZAP XR	LP04
HTGA HTGB HTGC HTGD	Apoptotic T-cell	Uni-ZAP XR	LP04
HMCA HMCB HMCC HMCD HMCE	Macrophage-oxLDL	Uni-ZAP XR	LP04
HMAA HMAB HMAC HMAD HMAE HMAF HMAG	Macrophage (GM-CSF treated)	Uni-ZAP XR	LP04
HPHA	Normal Prostate	Uni-ZAP XR	LP04
HPIA HPIB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP04
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP04
HOSE HOSF HOSG	Human Osteoclastoma, re-excision	Uni-ZAP XR	LP04
HTGE HTGF	Apoptotic T-cell, re-excision	Uni-ZAP XR	LP04
HMAJ HMAK	H Macrophage (GM-CSF treated), re-excision	Uni-ZAP XR	LP04
HACB HACC HACD	Human Adipose Tissue, re-excision	Uni-ZAP XR	LP04
HFPA	H. Frontal Cortex, Epileptic	Uni-ZAP XR	LP04
HFAA HFAB HFAC HFAD HFAE	Alzheimers, spongy change	Uni-ZAP XR	LP04
HFAM	Frontal Lobe, Dementia	Uni-ZAP XR	LP04
HMIA HMIB HMIC	Human Manic Depression Tissue	Uni-ZAP XR	LP04
HTSA HTSE HTSF HTSG HTSH	Human Thymus	pBS	LP05
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBS	LP05
HSAA HSAB HSAC	HSA 172 Cells	pBS	LP05
HSBA HSBB HSBC HSBM	HSC172 cells	pBS	LP05
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBS	LP05
HJBA HJBB HJBC HJBD	Jurkat T-Cell, S phase	pBS	LP05
HAFA HAFB	Aorta endothelial cells + TNF-a	pBS	LP05
HAWA HAWB HAWC	Human White Adipose	pBS	LP05
HTNA HTNB	Human Thyroid	pBS	LP05
HONA	Normal Ovary, Premenopausal	pBS	LP05
HARA HARB	Human Adult Retina	pBS	LP05

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HLJA HLJB	Human Lung	pCMVSport 1	LP06
HOFM HOFN HOFO	H. Ovarian Tumor. II. OV5232	pCMVSport 2.0	LP07
HOGA HOGB HOGC	OV 10-3-95	pCMVSport 2.0	LP07
HCGL	CD34+cells. II	pCMVSport 2.0	LP07
HDLA	Hodgkin's Lymphoma I	pCMVSport 2.0	LP07
HDTA HDTB HDTC HDTD HDTE	Hodgkin's Lymphoma II	pCMVSport 2.0	LP07
HKAA HKAB HKAC HKAD HKAE HKAF HKAG HKAH	Keratinocyte	pCMVSport2.0	LP07
HCIM	CAPFINDER. Crohn's Disease. lib 2	pCMVSport 2.0	LP07
HKAL	Keratinocyte. lib 2	pCMVSport2.0	LP07
HKAT	Keratinocyte. lib 3	pCMVSport2.0	LP07
HNDA	Nasal polyps	pCMVSport2.0	LP07
HDRA	H. Primary Dendritic Cells.lib 3	pCMVSport2.0	LP07
HOHA HOHB HOHC	Human Osteoblasts II	pCMVSport2.0	LP07
HLDA HLDB HLDC	Liver. Hepatoma	pCMVSport3.0	LP08
HLDN HLDO HLDP	Human Liver. normal	pCMVSport3.0	LP08
HMTA	pBMC stimulated w/ poly I/C	pCMVSport3.0	LP08
HNTA	NTERA2. control	pCMVSport3.0	LP08
HDPA HDPB HDPC HDPD HDPF HDPG HDPH HDPI HDPJ HDPK	Primary Dendritic Cells. lib 1	pCMVSport3.0	LP08
HDPM HDPN HDPO HDPP	Primary Dendritic cells.frac 2	pCMVSport3.0	LP08
HMUA HMUB HMUC	Myoloid Progenitor Cell Line	pCMVSport3.0	LP08
HHEA HHEB HHEC HHED	T Cell helper I	pCMVSport3.0	LP08
HHEM HHEN HHEO HHEP	T cell helper II	pCMVSport3.0	LP08
HEQA HEQB HEQC	Human endometrial stromal cells	pCMVSport3.0	LP08
HJMA HJMB	Human endometrial stromal cells-treated with progesterone	pCMVSport3.0	LP08
HSWA HSWB HSWC	Human endometrial stromal cells-treated with estradiol	pCMVSport3.0	LP08
HSYA HSYB HSYC	Human Thymus Stromal Cells	pCMVSport3.0	LP08
HLWA HLWB HLWC	Human Placenta	pCMVSport3.0	LP08
HRAA HRAB HRAC	Rejected Kidney. lib 4	pCMVSport3.0	LP08
HMTM	PCR. pBMC I/C treated	PCRII	LP09
HMJA	H. Meniingima. M6	pSport 1	LP10
HMKA HMKB HMKC HMKD HMKE	H. Meningima. M1	pSport 1	LP10
HUSG HUSI	Human umbilical vein endothelial cells. IL-4 induced	pSport 1	LP10
HUSX HUSY	Human Umbilical Vein Endothelial Cells. uninduced	pSport 1	LP10
HOFA	Ovarian Tumor I. OV5232	pSport 1	LP10
HCFA HCFB HCFC HCFD	T-Cell PHA 16 hrs	pSport 1	LP10
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport 1	LP10
HADA HADC HADD HADE HADF HADG	Human Adipose	pSport 1	LP10
HOVA HOVB HOVC	Human Ovary	pSport 1	LP10
HTWB HTWC HTWD HTWE HTWF	Resting T-Cell Library.II	pSport 1	LP10

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMMA	Spleen metastic melanoma	pSport 1	LP10
HLYA HLYB HLYC HLYD HLYE	Spleen. Chronic lymphocytic leukemia	pSport 1	LP10
HCGA	CD34+ cell. I	pSport 1	LP10
HEOM HEON	Human Eosinophils	pSport 1	LP10
HTDA	Human Tonsil. Lib 3	pSport 1	LP10
HSPA	Salivary Gland. Lib 2	pSport 1	LP10
HCHA HCHB HCHC	Breast Cancer cell line. MDA 36	pSport 1	LP10
HCHM HCHN	Breast Cancer Cell line, angiogenic	pSport 1	LP10
HCIA	Crohn's Disease	pSport 1	LP10
HDAA HDAB HDAC	HEL cell line	pSport 1	LP10
HABA	Human Astrocyte	pSport 1	LP10
HUFA HUFB HUFC	Ulcerative Colitis	pSport 1	LP10
HNTM	NTERA2 + retinoic acid. 14 days	pSport 1	LP10
HDQA	Primary Dendritic cells.CapFinder2. frac 1	pSport 1	LP10
HDQM	Primary Dendritic Cells. CapFinder. frac 2	pSport 1	LP10
HLDX	Human Liver. normal.CapFinder	pSport 1	LP10
HULA HULB HULC	Human Dermal Endothelial Cells.untreated	pSport1	LP10
HUMA	Human Dermal Endothelial cells.treated	pSport1	LP10
HCJA	Human Stromal Endometrial fibroblasts, untreated	pSport1	LP10
HCJM	Human Stromal endometrial fibroblasts, treated w/ estradiol	pSport1	LP10
HEDA	Human Stromal endometrial fibroblasts, treated with progesterone	pSport1	LP10
HFNA	Human ovary tumor cell OV350721	pSport1	LP10
HKGA HKGB HKGC HKGD	Merkel Cells	pSport1	LP10
HISA HISB HISC	Pancreas Islet Cell Tumor	pSport1	LP10
HLSA	Skin. burned	pSport1	LP10
HBZA	Prostate.BPH. Lib 2	pSport 1	LP10
HBZS	Prostate BPH.Lib 2. subtracted	pSport 1	LP10
HFIA HFIB HFIC	Synovial Fibroblasts (control)	pSport 1	LP10
HFIH HFII HFIJ	Synovial hypoxia	pSport 1	LP10
HFIT HFIU HFIV	Synovial IL-1/TNF stimulated	pSport 1	LP10
HGCA	Messangial cell. frac 1	pSport1	LP10
HMVA HMVB HMVC	Bone Marrow Stromal Cell. untreated	pSport1	LP10
HFIX HFIY HFIZ	Synovial Fibroblasts (Il1/TNF). subt	pSport1	LP10
HFOX HFOY HFOZ	Synovial hypoxia-RSF subtracted	pSport1	LP10
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP11
HLIA HLIB HLIC	Human Liver	pCMVSport 1	LP012
HHBA HHBB HHBC HHBD HHBE	Human Heart	pCMVSport 1	LP012
HBBA HBBB	Human Brain	pCMVSport 1	LP012
HLJA HLJB HLJC HLJD HLJE	Human Lung	pCMVSport 1	LP012
HOGA HOGB HOGC	Ovarian Tumor	pCMVSport 2.0	LP012

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HTJM	Human Tonsils. Lib 2	pCMVSport 2.0	LP012
HAMF HAMG	KMH2	pCMVSport 3.0	LP012
HAJA HAJB HAJC	L428	pCMVSport 3.0	LP012
HWBA HWBB HWBC HWBD HWBE	Dendritic cells. pooled	pCMVSport 3.0	LP012
HWAA HWAB HWAC HWAD HWAE	Human Bone Marrow. treated	pCMVSport 3.0	LP012
HYAA HYAB HYAC	B Cell lymphoma	pCMVSport 3.0	LP012
HWHG HWHH HWHI	Healing groin wound, 6.5 hours post incision	pCMVSport 3.0	LP012
HWHP HWHQ HWHR	Healing groin wound: 7.5 hours post incision	pCMVSport 3.0	LP012
HARM	Healing groin wound - zero hr post-incision (control)	pCMVSport 3.0	LP012
HBIM	Olfactory epithelium: nasalcavity	pCMVSport 3.0	LP012
HWDA	Healing Abdomen wound: 70&90 min post incision	pCMVSport 3.0	LP012
HWEA	Healing Abdomen Wound:15 days post incision	pCMVSport 3.0	LP012
HWJA	Healing Abdomen Wound:21&29 days	pCMVSport 3.0	LP012
HNAL	Human Tongue. frac 2	pSport1	LP012
HMJA	H. Meniingima. M6	pSport1	LP012
HMKA HMKB HMKC HMKD HMKE	H. Meningima. M1	pSport1	LP012
HOFA	Ovarian Tumor I. OV5232	pSport1	LP012
HCFA HCFB HCFC HCFD	T-Cell PHA 16 hrs	pSport1	LP012
HCFL HCFM HCFN HCFO	T-Cell PHA 24 hrs	pSport1	LP012
HMMA HMMB HMMC	Spleen metastic melanoma	pSport1	LP012
HTDA	Human Tonsil. Lib 3	pSport1	LP012
HDBA	Human Fetal Thymus	pSport1	LP012
HDUA	Pericardium	pSport1	LP012
HBZA	Prostate.BPH. Lib 2	pSport1	LP012
HWCA	Larynx tumor	pSport1	LP012
HWKA	Normal lung	pSport1	LP012
HSMB	Bone marrow stroma.treated	pSport1	LP012
HBHM	Normal trachea	pSport1	LP012
HLFC	Human Larynx	pSport1	LP012
HLRB	Siebben Polyposis	pSport1	LP012
HNIA	Mammary Gland	pSport1	LP012
HNJB	Palate carcinoma	pSport1	LP012
HNKA	Palate normal	pSport1	LP012
HMZA	Pharynx carcinoma	pSport1	LP012
HABG	Cheek Carcinoma	pSport1	LP012
HMZM	Pharynx Carcinoma	pSport1	LP012
HDRM	Larynx Carcinoma	pSport1	LP012
HVAA	Pancreas normal PCA4 No	pSport1	LP012
HICA	Tongue carcinoma	pSport1	LP012
HUKA HUKB HUKC HUKD HUKE	Human Uterine Cancer	Lambda ZAP II	LP013
HFFA	Human Fetal Brain. random primed	Lambda ZAP II	LP013
HTUA	Activated T-cell labeled with 4-thioluri	Lambda ZAP II	LP013
HBQA	Early Stage Human Brain. random primed	Lambda ZAP II	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HMEB	Human microvascular Endothelial cells, fract. B	Lambda ZAP II	LP013
HUSH	Human Umbilical Vein Endothelial cells, fract. A, re-excision	Lambda ZAP II	LP013
HLQC HLQD	Hepatocellular tumor, re-excision	Lambda ZAP II	LP013
HTWJ HTWK HTWL	Resting T-cell, re-excision	Lambda ZAP II	LP013
HF6S	Human Whole 6 week Old Embryo (II), subt	pBluescript	LP013
HHPS	Human Hippocampus, subtracted	pBluescript	LP013
HL1S	LNCAP, differential expression	pBluescript	LP013
HLHS HLHT	Early Stage Human Lung, Subtracted	pBluescript	LP013
HSUS	Supt cells, cyclohexamide treated, subtracted	pBluescript	LP013
HSUT	Supt cells, cyclohexamide treated, differentially expressed	pBluescript	LP013
HSDS	H. Striatum Depression, subtracted	pBluescript	LP013
HPTZ	Human Pituitary, Subtracted VII	pBluescript	LP013
HSDX	H. Striatum Depression, subt II	pBluescript	LP013
HSDZ	H. Striatum Depression, subt	pBluescript	LP013
HPBA HPBB HPBC HPBD HPBE	Human Pineal Gland	pBluescript SK-	LP013
HRTA	Colorectal Tumor	pBluescript SK-	LP013
HSBA HSBB HSBC HSBM	HSC172 cells	pBluescript SK-	LP013
HJAA HJAB HJAC HJAD	Jurkat T-cell G1 phase	pBluescript SK-	LP013
HJBA HJBB HJBC HJBD	Jurkat T-cell, S1 phase	pBluescript SK-	LP013
HTNA HTNB	Human Thyroid	pBluescript SK-	LP013
HAHA HAHB	Human Adult Heart	Uni-ZAP XR	LP013
HE6A	Whole 6 week Old Embryo	Uni-ZAP XR	LP013
HFCA HFCB HFCC HFCD HFCE	Human Fetal Brain	Uni-ZAP XR	LP013
HFKC HFKD HFKE HFKF HFKG	Human Fetal Kidney	Uni-ZAP XR	LP013
HGBA HGBD HGBE HGBF HGBG	Human Gall Bladder	Uni-ZAP XR	LP013
HPRA HPRB HPRC HPRD	Human Prostate	Uni-ZAP XR	LP013
HTEA HTEB HTEC HTED HTEE	Human Testes	Uni-ZAP XR	LP013
HTTA HTTB HTTC HTTD HTTE	Human Testes Tumor	Uni-ZAP XR	LP013
HYBA HYBB	Human Fetal Bone	Uni-ZAP XR	LP013
HFLA	Human Fetal Liver	Uni-ZAP XR	LP013
HHFB HHFC HHFD HHFE HHFF	Human Fetal Heart	Uni-ZAP XR	LP013
HUVB HUVC HUVD HUVE	Human Umbilical Vein, End. remake	Uni-ZAP XR	LP013
HTHB HTHC HTHD	Human Thymus	Uni-ZAP XR	LP013
HSTA HSTB HSTC HSTD	Human Skin Tumor	Uni-ZAP XR	LP013
HTAA HTAB HTAC HTAD HTAE	Human Activated T-cells	Uni-ZAP XR	LP013
HFEA HFEB HFEC	Human Fetal Epithelium (skin)	Uni-ZAP XR	LP013
HJPA HJPB HJPC HJPD	Human Jurkat Membrane Bound Polysomes	Uni-ZAP XR	LP013
HESA	Human Epithelioid Sarcoma	Uni-ZAP XR	LP013
HALS	Human Adult Liver, Subtracted	Uni-ZAP XR	LP013
HFTA HFTB HFTC HFTD	Human Fetal Dura Mater	Uni-ZAP XR	LP013
HCAA HCAB HCAC	Cem cells, cyclohexamide treated	Uni-ZAP XR	LP013
HRGA HRGB HRGC HRGD	Raji Cells, cyclohexamide treated	Uni-ZAP XR	LP013
HE9A HE9B HE9C HE9D HE9E	Nine Week Old Early Stage Human	Uni-ZAP XR	LP013

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HSFA	Human Fibrosarcoma	Uni-ZAP XR	LP013
HATA HATB HATC HATD HATE	Human Adrenal Gland Tumor	Uni-ZAP XR	LP013
HTRA	Human Trachea Tumor	Uni-ZAP XR	LP013
HE2A HE2D HE2E HE2H HE2I	12 Week Old Early Stage Human	Uni-ZAP XR	LP013
HE2B HE2C HE2F HE2G HE2P	12 Week Old Early Stage Human, II	Uni-ZAP XR	LP013
HNEA HNEB HNEC HNED HNEE	Human Neutrophil	Uni-ZAP XR	LP013
HBGA	Human Primary Breast Cancer	Uni-ZAP XR	LP013
HPTS HPTT HPTU	Human Pituitary, subtracted	Uni-ZAP XR	LP013
HMQA HMQB HMQC HMQD	Human Activated Monocytes	Uni-ZAP XR	LP013
HOAA HOAB HOAC	Human Osteosarcoma	Uni-ZAP XR	LP013
HTOA HTOD HTOE HTOF HTOG	human tonsils	Uni-ZAP XR	LP013
HMGB	Human OB MG63 control fraction I	Uni-ZAP XR	LP013
HOPB	Human OB HOS control fraction I	Uni-ZAP XR	LP013
HOQB	Human OB HOS treated (1 nM E2) fraction I	Uni-ZAP XR	LP013
HAUA HAUB HAUC	Amniotic Cells - TNF induced	Uni-ZAP XR	LP013
HAQA HAQB HAQC HAQD	Amniotic Cells - Primary Culture	Uni-ZAP XR	LP013
HROA HROC	HUMAN STOMACH	Uni-ZAP XR	LP013
HBJA HBJB HBJC HBJD HBJE	HUMAN B CELL LYMPHOMA	Uni-ZAP XR	LP013
HODA HODB HODC HODD	human ovarian cancer	Uni-ZAP XR	LP013
HCPA	Corpus Callosum	Uni-ZAP XR	LP013
HSOA	stomach cancer (human)	Uni-ZAP XR	LP013
HERA	SKIN	Uni-ZAP XR	LP013
HMDA	Brain-medulloblastoma	Uni-ZAP XR	LP013
HGLA HGLB HGLD	Glioblastoma	Uni-ZAP XR	LP013
HWTA HWTB HWTC	wilm's tumor	Uni-ZAP XR	LP013
HEAA	H. Atrophic Endometrium	Uni-ZAP XR	LP013
HAPN HAPO HAPP HAPQ HAPR	Human Adult Pulmonary;re-excision	Uni-ZAP XR	LP013
HLTG HLTH	Human T-cell lymphoma;re-excision	Uni-ZAP XR	LP013
HAHC HAHD HAHE	Human Adult Heart;re-excision	Uni-ZAP XR	LP013
HAGA HAGB HAGC HAGD HAGE	Human Amygdala	Uni-ZAP XR	LP013
HSJA HSJB HSJC	Smooth muscle-ILb induced	Uni-ZAP XR	LP013
HSHA HSHB HSHC	Smooth muscle, IL1b induced	Uni-ZAP XR	LP013
HPWA HPWB HPWC HPWD HPWE	Prostate BPH	Uni-ZAP XR	LP013
HPIA HPIB HPIC	LNCAP prostate cell line	Uni-ZAP XR	LP013
HPJA HPJB HPJC	PC3 Prostate cell line	Uni-ZAP XR	LP013
HBTA	Bone Marrow Stroma, TNF&LPS ind	Uni-ZAP XR	LP013
HMCF HMCG HMCH HMCI HMCJ	Macrophage-oxLDL; re-excision	Uni-ZAP XR	LP013
HAGG HAGH HAGI	Human Amygdala;re-excision	Uni-ZAP XR	LP013
HACA	H. Adipose Tissue	Uni-ZAP XR	LP013
HKFB	K562 + PMA (36 hrs),re-excision	ZAP Express	LP013
HCWT HCWU HCWV	CD34 positive cells (cord blood),re-ex	ZAP Express	LP013
HBWA	Whole brain	ZAP Express	LP013
HBXA HBXB HBXC HBXD	Human Whole Brain #2 - Oligo dT > 1.5Kb	ZAP Express	LP013
HAVM	Temporal cortex-Alzheizmer	pT-Adv	LP014
HAVT	Hippocampus, Alzheimer Subtracted	pT-Adv	LP014

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HHAS	CHME Cell Line	Uni-ZAP XR	LP014
HAJR	Larynx normal	pSport 1	LP014
HWLE HWLF HWLG HWLH	Colon Normal	pSport 1	LP014
HCRM HCRN HCRO	Colon Carcinoma	pSport 1	LP014
HWLI HWLJ HWLK	Colon Normal	pSport 1	LP014
HWLQ HWLR HWLS HWLT	Colon Tumor	pSport 1	LP014
HBFM	Gastrocnemius Muscle	pSport 1	LP014
HBOD HBOE	Quadriceps Muscle	pSport 1	LP014
HBKD HBKE	Soleus Muscle	pSport 1	LP014
HCCM	Pancreatic Langerhans	pSport 1	LP014
HWGA	Larynx carcinoma	pSport 1	LP014
HWGM HWGN	Larynx carcinoma	pSport 1	LP014
HWLA HWLB HWLC	Normal colon	pSport 1	LP014
HWLM HWLN	Colon Tumor	pSport 1	LP014
HVAM HVAN HVAO	Pancreas Tumor	pSport 1	LP014
HWGQ	Larynx carcinoma	pSport 1	LP014
HAQM HAQN	Salivary Gland	pSport 1	LP014
HASM	Stomach; normal	pSport 1	LP014
HBCM	Uterus; normal	pSport 1	LP014
HCDM	Testis; normal	pSport 1	LP014
HDJM	Brain; normal	pSport 1	LP014
HEFM	Adrenal Gland,normal	pSport 1	LP014
HBAA	Rectum normal	pSport 1	LP014
HFDM	Rectum tumour	pSport 1	LP014
HGAM	Colon, normal	pSport 1	LP014
HHMM	Colon, tumour	pSport 1	LP014
HCLB HCLC	Human Lung Cancer	Lambda Zap II	LP015
HRLA	L1 Cell line	ZAP Express	LP015
HHAM	Hypothalamus, Alzheimer's	pCMVSport 3.0	LP015
HKBA	Ku 812F Basophils Line	pSport 1	LP015
HS2S	Saos2, Dexamethosome Treated	pSport 1	LP016
HASA	Lung Carcinoma A549 TNFalpha activated	pSport 1	LP016
HTFM	TF-1 Cell Line GM-CSF Treated	pSport 1	LP016
HYAS	Thyroid Tumour	pSport 1	LP016
HUTS	Larynx Normal	pSport 1	LP016
HXOA	Larynx Tumor	pSport 1	LP016
HEAH	Ea.hy.926 cell line	pSport 1	LP016
HINA	Adenocarcinoma Human	pSport 1	LP016
HRMA	Lung Mesothelium	pSport 1	LP016
HLCL	Human Pre-Differentiated Adipocytes	Uni-Zap XR	LP017
HS2A	Saos2 Cells	pSport 1	LP020
HS2I	Saos2 Cells; Vitamin D3 Treated	pSport 1	LP020
HUCM	CHME Cell Line, untreated	pSport 1	LP020
HEPN	Aryepiglottis Normal	pSport 1	LP020
HPSN	Sinus Piniformis Tumour	pSport 1	LP020
HNSA	Stomach Normal	pSport 1	LP020

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HNSM	Stomach Tumour	pSport 1	LP020
HNLA	Liver Normal Met5No	pSport 1	LP020
HUTA	Liver Tumour Met 5 Tu	pSport 1	LP020
HOCN	Colon Normal	pSport 1	LP020
HOCT	Colon Tumor	pSport 1	LP020
HTNT	Tongue Tumour	pSport 1	LP020
HLXN	Larynx Normal	pSport 1	LP020
HLXT	Larynx Tumour	pSport 1	LP020
HTYN	Thymus	pSport 1	LP020
HPLN	Placenta	pSport 1	LP020
HTNG	Tongue Normal	pSport 1	LP020
HZAA	Thyroid Normal (SDCA2 No)	pSport 1	LP020
HWES	Thyroid Thyroiditis	pSport 1	LP020
HFHD	Ficolled Human Stromal Cells. 5Fu treated	pTrip1Ex2	LP021
HFHM.HFHN	Ficolled Human Stromal Cells. Untreated	pTrip1Ex2	LP021
HPCI	Hep G2 Cells. lambda library	lambda Zap-CMV XR	LP021
HBCA.HBCB.HBCC	H. Lymph node breast Cancer	Uni-ZAP XR	LP021
HCOK	Chondrocytes	pSPORT1	LP022
HDCA. HDCB. HDCC	Dendritic Cells From CD34 Cells	pSPORT1	LP022
HDMA. HDMB	CD40 activated monocyte dendritic cells	pSPORT1	LP022
HDDM. HDDN. HDDO	LPS activated derived dendritic cells	pSPORT1	LP022
HPCR	Hep G2 Cells. PCR library	lambda Zap-CMV XR	LP022
HAAA. HAAB. HAAC	Lung, Cancer (4005313A3): Invasive Poorly Differentiated Lung Adenocarcinoma	pSPORT1	LP022
HIPA. HIPB, HIPC	Lung, Cancer (4005163 B7): Invasive. Poorly Diff. Adenocarcinoma. Metastatic	pSPORT1	LP022
HOOH. HOOI	Ovary. Cancer: (4004562 B6) Papillary Serous Cystic Neoplasm. Low Malignant Pot	pSPORT1	LP022
HIDA	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HUJA.HUJB.HUJC.HUJD.HUJE	B-Cells	pCMVSport 3.0	LP022
HNOA.HNOB.HNOC.HNOD	Ovary. Normal: (9805C040R)	pSPORT1	LP022
HNLM	Lung, Normal: (4005313 B1)	pSPORT1	LP022
HSCL	Stromal Cells	pSPORT1	LP022
HAAX	Lung, Cancer: (4005313 A3) Invasive Poorly-differentiated Metastatic lung adenocarcinoma	pSPORT1	LP022
HUUA.HUUB.HUUC.HUUD	B-cells (unstimulated)	pTrip1Ex2	LP022
HWWA.HWWB.HWWC.HWWD.HWWE.HWWF.HWWG	B-cells (stimulated)	pSPORT1	LP022
HCCC	Colon. Cancer: (9808C064R)	pCMVSport 3.0	LP023
HPDO HPDP HPDQ HPDR HPD	Ovary. Cancer (9809C332): Poorly differentiated adenocarcinoma	pSport 1	LP023
HPCO HPCP HPCQ HPCT	Ovary, Cancer (15395A1F): Grade II Papillary Carcinoma	pSport 1	LP023
HOCM HOCO HOCP HOCQ	Ovary, Cancer: (15799A1F) Poorly differentiated carcinoma	pSport 1	LP023

Libraries owned by Catalog	Catalog Description	Vector	ATCC Deposit
HCBM HCBN HCBO	Breast. Cancer: (4004943 A5)	pSport 1	LP023
HNBT HNBU HNBV	Breast. Normal: (4005522B2)	pSport 1	LP023
HBCP HBCQ	Breast. Cancer: (4005522 A2)	pSport 1	LP023
HBCJ	Breast. Cancer: (9806C012R)	pSport 1	LP023
HSAM HSAN	Stromal cells 3.88	pSport 1	LP023
HVCA HVCB HVCC HVCD	Ovary. Cancer: (4004332 A2)	pSport 1	LP023
HSCK HSEN HSEO	Stromal cells (HBM3.18)	pSport 1	LP023
HSCP HSCQ	stromal cell clone 2.5	pSport 1	LP023
HUXA	Breast Cancer: (4005385 A2)	pSport 1	LP023
HCOM HCON HCOO HCOP HCOQ	Ovary. Cancer (4004650 A3): Well-Differentiated Micropapillary Serous Carcinoma	pSport 1	LP023
HBNM	Breast. Cancer: (9802C020E)	pSport 1	LP023
HVVA HVVB HVVC HVVD HVVE	Human Bone Marrow. treated	pSport 1	LP023

Two approaches can be used to isolate a particular clone from the deposited sample of plasmid DNAs cited for that clone in Table 5. First, a plasmid is directly isolated by screening the clones using a polynucleotide probe corresponding to the nucleotide sequence of SEQ ID NO:X.

Particularly, a specific polynucleotide with 30-40 nucleotides is synthesized using an Applied Biosystems DNA synthesizer according to the sequence reported. The oligonucleotide is labeled, for instance, with ^{32}P-γ-ATP using T4 polynucleotide kinase and purified according to routine methods. (E.g., Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring, NY (1982).) The plasmid mixture is transformed into a suitable host, as indicated above (such as XL-1 Blue (Stratagene)) using techniques known to those of skill in the art, such as those provided by the vector supplier or in related publications or patents cited above. The transformants are plated on 1.5% agar plates (containing the appropriate selection agent, e.g., ampicillin) to a density of about 150 transformants (colonies) per plate. These plates are screened using Nylon membranes according to routine methods for bacterial colony screening (e.g., Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Edit., (1989), Cold Spring Harbor Laboratory Press, pages 1.93 to 1.104), or other techniques known to those of skill in the art.

Alternatively, two primers of 17-20 nucleotides derived from both ends of the nucleotide sequence of SEQ ID NO:X are synthesized and used to amplify the desired cDNA using the deposited cDNA plasmid as a template. The polymerase chain reaction is carried out under routine conditions, for instance, in 25 μl of reaction mixture with 0.5 ug of the above cDNA template. A convenient reaction mixture is 1.5-5 mM $MgCl_2$, 0.01% (w/v) gelatin, 20 μM each of dATP, dCTP, dGTP, dTTP, 25 pmol of each primer and 0.25 Unit of Taq polymerase. Thirty five cycles of PCR (denaturation at 94°C for 1 min; annealing at 55°C for 1 min; elongation at 72°C for 1 min) are performed with a Perkin-Elmer Cetus automated thermal cycler. The amplified product is analyzed by agarose gel electrophoresis and the DNA band with expected molecular weight is excised and purified. The PCR product is verified to be the selected sequence by subcloning and sequencing the DNA product.

Several methods are available for the identification of the 5' or 3' non-coding portions of a gene which may not be present in the deposited clone. These methods include but are not limited to, filter probing, clone enrichment using specific probes, and protocols similar or identical to 5' and 3' "RACE" protocols which are well known in the art. For instance, a method similar to 5' RACE is available for generating the missing 5' end of a desired full-length transcript. (Fromont-Racine et al., Nucleic Acids Res. 21(7):1683-1684 (1993).)

Briefly, a specific RNA oligonucleotide is ligated to the 5' ends of a population of RNA presumably containing full-length gene RNA transcripts. A primer set containing a primer specific to the ligated RNA oligonucleotide and a primer specific to a known sequence of the gene of interest is used to PCR amplify the 5' portion of the desired full-length gene. This amplified product may then be sequenced and used to generate the full length gene.

This above method starts with total RNA isolated from the desired source, although poly-A+ RNA can be used. The RNA preparation can then be treated with phosphatase if necessary to eliminate 5' phosphate groups on degraded or damaged RNA which may interfere with the later RNA ligase step. The phosphatase should then be inactivated and the RNA treated with tobacco acid pyrophosphatase in order to remove the cap structure present at the 5' ends of messenger RNAs. This reaction leaves a 5' phosphate group at the 5' end of the cap cleaved RNA which can then be ligated to an RNA oligonucleotide using T4 RNA ligase.

This modified RNA preparation is used as a template for first strand cDNA synthesis using a gene specific oligonucleotide. The first strand synthesis reaction is used as a template for PCR amplification of the desired 5' end using a primer specific to the ligated RNA oligonucleotide and a primer specific to the known sequence of the gene of interest. The resultant product is then sequenced and analyzed to confirm that the 5' end sequence belongs to the desired gene.

Example 2: Isolation of Genomic Clones Corresponding to a Polynucleotide

A human genomic P1 library (Genomic Systems, Inc.) is screened by PCR using primers selected for the sequence corresponding to SEQ ID NO:X, according to the method described in Example 1. (See also, Sambrook.)

Example 3: Tissue specific expression analysis

The Human Genome Sciences, Inc. (HGS) database is derived from sequencing tissue specific cDNA libraries. Libraries generated from a particular tissue are selected and the specific tissue expression pattern of EST groups or assembled contigs within these libraries is determined by comparison of the expression patterns of those groups or contigs within the entire database. ESTs which show tissue specific expression are selected.

The original clone from which the specific EST sequence was generated, is obtained from the catalogued library of clones and the insert amplified by PCR using methods known in the art. The PCR product is denatured then transferred in 96 well format to a nylon membrane (Schleicher and Scheull) generating an array filter of tissue specific clones. Housekeeping genes, maize genes, and known tissue specific genes are included on the filters. These targets can be used in signal normalization and to validate assay sensitivity. Additional targets are included to monitor probe length and specificity of hybridization.

Radioactively labeled hybridization probes are generated by first strand cDNA synthesis per the manufacturer's instructions (Life Technologies) from mRNA/RNA samples prepared from the specific tissue being analyzed. The hybridization probes are purified by gel exclusion chromatography, quantitated, and hybridized with the array filters in hybridization bottles at 65°C overnight. The filters are washed under stringent conditions and signals are captured using a Fuji phosphorimager.

Data is extracted using AIS software and following background subtraction, signal normalization is performed. This includes a normalization of filter-wide

expression levels between different experimental runs. Genes that are differentially expressed in the tissue of interest are identified and the full length sequence of these clones is generated.

Example 4: Chromosomal Mapping of the Polynucleotides

An oligonucleotide primer set is designed according to the sequence at the 5' end of SEQ ID NO:X. This primer preferably spans about 100 nucleotides. This primer set is then used in a polymerase chain reaction under the following set of conditions : 30 seconds, 95°C; 1 minute, 56°C; 1 minute, 70°C. This cycle is repeated 32 times followed by one 5 minute cycle at 70°C. Human, mouse, and hamster DNA is used as template in addition to a somatic cell hybrid panel containing individual chromosomes or chromosome fragments (Bios, Inc). The reactions is analyzed on either 8% polyacrylamide gels or 3.5 % agarose gels. Chromosome mapping is determined by the presence of an approximately 100 bp PCR fragment in the particular somatic cell hybrid.

Example 5: Bacterial Expression of a Polypeptide

A polynucleotide encoding a polypeptide of the present invention is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, as outlined in Example 1, to synthesize insertion fragments. The primers used to amplify the cDNA insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a

ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl by stirring for 3-4 hours at 4°C. The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA") affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8, the column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the protein can be successfully refolded while immobilized on the Ni-NTA column. The recommended conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM immidazole. Immidazole is removed by a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

In addition to the above expression vector, the present invention further includes an expression vector comprising phage operator and promoter elements operatively linked to a polynucleotide of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter sequence and operator sequences are made synthetically.

DNA can be inserted into the pHEa by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is generated according to the PCR protocol described in Example 1, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector could easily be substituted in the above protocol to express protein in a bacterial system.

Example 6: Purification of a Polypeptide from an Inclusion Body

The following alternative method can be used to purify a polypeptide expressed in *E coli* when it is present in the form of inclusion bodies. Unless otherwise specified, all of the following steps are conducted at 4-10°C.

Upon completion of the production phase of the *E. coli* fermentation, the cell culture is cooled to 4-10°C and the cells harvested by continuous centrifugation at 15,000 rpm (Heraeus Sepatech). On the basis of the expected yield of protein per unit weight of cell paste and the amount of purified protein required, an appropriate amount of cell paste, by weight, is suspended in a buffer solution containing 100 mM Tris, 50 mM EDTA, pH 7.4. The cells are dispersed to a homogeneous suspension using a high shear mixer.

The cells are then lysed by passing the solution through a microfluidizer (Microfuidics, Corp. or APV Gaulin, Inc.) twice at 4000-6000 psi. The homogenate is then mixed with NaCl solution to a final concentration of 0.5 M NaCl, followed by centrifugation at 7000 xg for 15 min. The resultant pellet is washed again using 0.5M NaCl, 100 mM Tris, 50 mM EDTA, pH 7.4.

The resulting washed inclusion bodies are solubilized with 1.5 M guanidine hydrochloride (GuHCl) for 2-4 hours. After 7000 xg centrifugation for 15 min., the pellet is discarded and the polypeptide containing supernatant is incubated at 4°C overnight to allow further GuHCl extraction.

Following high speed centrifugation (30,000 xg) to remove insoluble particles, the GuHCl solubilized protein is refolded by quickly mixing the GuHCl extract with 20 volumes of buffer containing 50 mM sodium, pH 4.5, 150 mM NaCl, 2 mM EDTA by vigorous stirring. The refolded diluted protein solution is kept at 4°C without mixing for 12 hours prior to further purification steps.

To clarify the refolded polypeptide solution, a previously prepared tangential filtration unit equipped with 0.16 μm membrane filter with appropriate surface area (e.g., Filtron), equilibrated with 40 mM sodium acetate, pH 6.0 is employed. The filtered sample is loaded onto a cation exchange resin (e.g., Poros HS-50, Perseptive

Biosystems). The column is washed with 40 mM sodium acetate, pH 6.0 and eluted with 250 mM, 500 mM, 1000 mM, and 1500 mM NaCl in the same buffer, in a stepwise manner. The absorbance at 280 nm of the effluent is continuously monitored. Fractions are collected and further analyzed by SDS-PAGE.

Fractions containing the polypeptide are then pooled and mixed with 4 volumes of water. The diluted sample is then loaded onto a previously prepared set of tandem columns of strong anion (Poros HQ-50, Perseptive Biosystems) and weak anion (Poros CM-20, Perseptive Biosystems) exchange resins. The columns are equilibrated with 40 mM sodium acetate, pH 6.0. Both columns are washed with 40 mM sodium acetate, pH 6.0, 200 mM NaCl. The CM-20 column is then eluted using a 10 column volume linear gradient ranging from 0.2 M NaCl, 50 mM sodium acetate, pH 6.0 to 1.0 M NaCl, 50 mM sodium acetate, pH 6.5. Fractions are collected under constant A_{280} monitoring of the effluent. Fractions containing the polypeptide (determined, for instance, by 16% SDS-PAGE) are then pooled.

The resultant polypeptide should exhibit greater than 95% purity after the above refolding and purification steps. No major contaminant bands should be observed from Commassie blue stained 16% SDS-PAGE gel when 5 μg of purified protein is loaded. The purified protein can also be tested for endotoxin/LPS contamination, and typically the LPS content is less than 0.1 ng/ml according to LAL assays.

Example 7: Cloning and Expression of a Polypeptide in a Baculovirus Expression System

In this example, the plasmid shuttle vector pA2 is used to insert a polynucleotide into a baculovirus to express a polypeptide. This expression vector contains the strong polyhedrin promoter of the *Autographa californica* nuclear polyhedrosis virus (AcMNPV) followed by convenient restriction sites such as BamHI, Xba I and Asp718. The polyadenylation site of the simian virus 40 ("SV40")

is used for efficient polyadenylation. For easy selection of recombinant virus, the plasmid contains the beta-galactosidase gene from *E. coli* under control of a weak Drosophila promoter in the same orientation, followed by the polyadenylation signal of the polyhedrin gene. The inserted genes are flanked on both sides by viral sequences for cell-mediated homologous recombination with wild-type viral DNA to generate a viable virus that express the cloned polynucleotide.

Many other baculovirus vectors can be used in place of the vector above, such as pAc373, pVL941, and pAcIM1, as one skilled in the art would readily appreciate, as long as the construct provides appropriately located signals for transcription, translation, secretion and the like, including a signal peptide and an in-frame AUG as required. Such vectors are described, for instance, in Luckow et al., Virology 170:31-39 (1989).

Specifically, the cDNA sequence contained in the deposited clone, including the AUG initiation codon, is amplified using the PCR protocol described in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the pA2 vector does not need a second signal peptide. Alternatively, the vector can be modified (pA2 GP) to include a baculovirus leader sequence, using the standard methods described in Summers et al., "A Manual of Methods for Baculovirus Vectors and Insect Cell Culture Procedures," Texas Agricultural Experimental Station Bulletin No. 1555 (1987).

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The plasmid is digested with the corresponding restriction enzymes and optionally, can be dephosphorylated using calf intestinal phosphatase, using routine procedures known in the art. The DNA is then isolated from a 1% agarose gel using a commercially available kit ("Geneclean" BIO 101 Inc., La Jolla, Ca.).

The fragment and the dephosphorylated plasmid are ligated together with T4 DNA ligase. *E. coli* HB101 or other suitable *E. coli* hosts such as XL-1 Blue

(Stratagene Cloning Systems. La Jolla, CA) cells are transformed with the ligation mixture and spread on culture plates. Bacteria containing the plasmid are identified by digesting DNA from individual colonies and analyzing the digestion product by gel electrophoresis. The sequence of the cloned fragment is confirmed by DNA sequencing.

Five μg of a plasmid containing the polynucleotide is co-transfected with 1.0 μg of a commercially available linearized baculovirus DNA ("BaculoGold™ baculovirus DNA", Pharmingen, San Diego, CA), using the lipofection method described by Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417 (1987). One μg of BaculoGold™ virus DNA and 5 μg of the plasmid are mixed in a sterile well of a microtiter plate containing 50 μl of serum-free Grace's medium (Life Technologies Inc., Gaithersburg, MD). Afterwards, 10 μl Lipofectin plus 90 μl Grace's medium are added, mixed and incubated for 15 minutes at room temperature. Then the transfection mixture is added drop-wise to Sf9 insect cells (ATCC CRL 1711) seeded in a 35 mm tissue culture plate with 1 ml Grace's medium without serum. The plate is then incubated for 5 hours at 27° C. The transfection solution is then removed from the plate and 1 ml of Grace's insect medium supplemented with 10% fetal calf serum is added. Cultivation is then continued at 27° C for four days.

After four days the supernatant is collected and a plaque assay is performed, as described by Summers and Smith, *supra*. An agarose gel with "Blue Gal" (Life Technologies Inc., Gaithersburg) is used to allow easy identification and isolation of gal-expressing clones, which produce blue-stained plaques. (A detailed description of a "plaque assay" of this type can also be found in the user's guide for insect cell culture and baculovirology distributed by Life Technologies Inc., Gaithersburg, page 9-10.) After appropriate incubation, blue stained plaques are picked with the tip of a micropipettor (e.g., Eppendorf). The agar containing the recombinant viruses is then resuspended in a microcentrifuge tube containing 200 μl of Grace's medium and the suspension containing the recombinant baculovirus is used to infect Sf9 cells seeded in 35 mm dishes. Four days later the supernatants of these culture dishes are harvested and then they are stored at 4° C.

To verify the expression of the polypeptide, Sf9 cells are grown in Grace's medium supplemented with 10% heat-inactivated FBS. The cells are infected with the recombinant baculovirus containing the polynucleotide at a multiplicity of infection ("MOI") of about 2. If radiolabeled proteins are desired, 6 hours later the medium is removed and is replaced with SF900 II medium minus methionine and cysteine (available from Life Technologies Inc., Rockville, MD). After 42 hours, 5 µCi of ^{35}S-methionine and 5 µCi ^{35}S-cysteine (available from Amersham) are added. The cells are further incubated for 16 hours and then are harvested by centrifugation. The proteins in the supernatant as well as the intracellular proteins are analyzed by SDS-PAGE followed by autoradiography (if radiolabeled).

Microsequencing of the amino acid sequence of the amino terminus of purified protein may be used to determine the amino terminal sequence of the produced protein.

Example 8: Expression of a Polypeptide in Mammalian Cells

The polypeptide of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

Suitable expression vectors for use in practicing the present invention include, for example, vectors such as pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC

67109), pCMVSport 2.0. and pCMVSport 3.0. Mammalian host cells that could be used include. human Hela. 293. H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the polypeptide can be expressed in stable cell lines containing the polynucleotide integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, hygromycin allows the identification and isolation of the transfected cells.

The transfected gene can also be amplified to express large amounts of the encoded protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt, F. W., et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin, J. L. and Ma, C., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page, M. J. and Sydenham, M. A., Biotechnology 9:64-68 (1991).) Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al., Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-enhancer (Boshart et al., Cell 41:521-530 (1985).) Multiple cloning sites, e.g., with the restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the gene of interest. The vectors also contain the 3' intron, the polyadenylation and termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate

restriction enzymes and then dephosphorylated using calf intestinal phosphates by procedures known in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide of the present invention is amplified according to the protocol outlined in Example 1. If a naturally occurring signal sequence is used to produce the polypeptide of the present invention, the vector does not need a second signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

The amplified fragment is isolated from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again purified on a 1% agarose gel.

The amplified fragment is then digested with the same restriction enzyme and purified on a 1% agarose gel. The isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase. *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme analysis.

Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five μg of the expression plasmid pC6 or pC4 is cotransfected with 0.5 μg of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of metothrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1 μM, 2 μM, 5 μM, 10 mM, 20 mM).

The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200 μM. Expression of the desired gene product is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

Example 9: Protein Fusions

The polypeptides of the present invention are preferably fused to other proteins. These fusion proteins can be used for a variety of applications. For example, fusion of the present polypeptides to His-tag, HA-tag, protein A, IgG domains. and maltose binding protein facilitates purification. (See Example 5: see also EP A 394,827; Traunecker. et al., Nature 331:84-86 (1988).) Similarly, fusion to IgG-1, IgG-3, and albumin increases the halflife time in vivo. Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of a fusion protein. Fusion proteins can also create chimeric molecules having more than one function. Finally, fusion proteins can increase solubility and/or stability of the fused protein compared to the non-fused protein. All of the types of fusion proteins described above can be made by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule, or the protocol described in Example 5.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian expression vector.

For example, if pC4 (Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide of the present invention, isolated by the PCR protocol described in Example 1, is ligated into this BamHI site. Note that the polynucleotide is cloned without a stop codon, otherwise a fusion protein will

not be produced.

If the naturally occurring signal sequence is used to produce the polypeptide of the present invention, pC4 does not need a second signal peptide. Alternatively, if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., WO 96/34891.)

Human IgG Fc region:
GGGATCCGGAGCCCAAATCTTCTGACAAAACTCACACATGCCCACCGTGC
CCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCCAAAA
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTCACATGCGTGGT
GGTGGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGG
ACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTA
CAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCA
ACCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAAC
CACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCAAGCGACATCGCCGT
GGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGACCACGCCT
CCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTG
GACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCA
TGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGG
GTAAATGAGTGCGACGGCCGCGACTCTAGAGGAT (SEQ ID NO:919)

Example 10: Production of an Antibody from a Polypeptide

a) Hybridoma Technology

The antibodies of the present invention can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, cells expressing polypeptide of the present invention are administered to an animal to induce the production of sera containing polyclonal antibodies. In a preferred method,

a preparation of polypeptide of the present invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for polypeptide of the present invention are prepared using hybridoma technology. (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with polypeptide of the present invention or, more preferably, with a secreted polypeptide of the present invention-expressing cell. Such polypeptide-expressing cells are cultured in any suitable tissue culture medium, preferably in Earle's modified Eagle's medium supplemented with 10% fetal bovine serum (inactivated at about 56°C), and supplemented with about 10 g/l of nonessential amino acids, about 1,000 U/ml of penicillin, and about 100 µg/ml of streptomycin.

The splenocytes of such mice are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding the polypeptide of the present invention.

Alternatively, additional antibodies capable of binding to polypeptide of the present invention can be produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method, protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify

clones which produce an antibody whose ability to bind to the polypeptide of the present invention-specific antibody can be blocked by polypeptide of the present invention. Such antibodies comprise anti-idiotypic antibodies to the polypeptide of the present invention-specific antibody and are used to immunize an animal to induce formation of further polypeptide of the present invention-specific antibodies.

For in vivo use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985).)

b) Isolation Of Antibody Fragments Directed Against Polypeptide of the Present Invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against polypeptide of the present invention to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

Rescue of the Library. A library of scFvs is constructed from the RNA of human PBLs as described in PCT publication WO 92/01047. To rescue phage displaying antibody fragments, approximately 109 E. coli harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 μg/ml of ampicillin (2xTY-AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to innoculate 50 ml of 2xTY-AMP-GLU, 2 x 108 TU of delta gene 3 helper (M13 delta gene III, see PCT publication WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet

resuspended in 2 liters of 2xTY containing 100 μg/ml ampicillin and 50 ug/ml kanamycin and grown overnight. Phage are prepared as described in PCT publication WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37° C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 μg ampicillin/ml and 25 μg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 μm filter (Minisart NML; Sartorius) to give a final concentration of approximately 1013 transducing units/ml (ampicillin-resistant clones).

Panning of the Library. Immunotubes (Nunc) are coated overnight in PBS with 4 ml of either 100 μg/ml or 10 μg/ml of a polypeptide of the present invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 1013 TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 μg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of selection. This process is then repeated for a total of 4 rounds of affinity purification

with tube-washing increased to 20 times with PBS. 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

Characterization of Binders. Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks. et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of the polypeptide of the present invention in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see. e.g., PCT publication WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example. epitope mapping. binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

Example 11: Method of Determining Alterations in a Gene Corresponding to a Polynucleotide

RNA isolated from entire families or individual patients presenting with a phenotype of interest (such as a disease) is be isolated. cDNA is then generated from these RNA samples using protocols known in the art. (See, Sambrook.) The cDNA is then used as a template for PCR, employing primers surrounding regions of interest in SEQ ID NO:X; and/or the nucleotide sequence of the related cDNA in the cDNA clone contained in a deposited library. Suggested PCR conditions consist of 35 cycles at 95 degrees C for 30 seconds; 60-120 seconds at 52-58 degrees C; and 60-120 seconds at 70 degrees C, using buffer solutions described in Sidransky et al., Science 252:706 (1991).

PCR products are then sequenced using primers labeled at their 5' end with T4 polynucleotide kinase. employing SequiTherm Polymerase. (Epicentre

Technologies). The intron-exon borders of selected exons is also determined and genomic PCR products analyzed to confirm the results. PCR products harboring suspected mutations is then cloned and sequenced to validate the results of the direct sequencing.

PCR products is cloned into T-tailed vectors as described in Holton et al., Nucleic Acids Research, 19:1156 (1991) and sequenced with T7 polymerase (United States Biochemical). Affected individuals are identified by mutations not present in unaffected individuals.

Genomic rearrangements are also observed as a method of determining alterations in a gene corresponding to a polynucleotide. Genomic clones isolated according to Example 2 are nick-translated with digoxigenindeoxy-uridine 5'-triphosphate (Boehringer Manheim), and FISH performed as described in Johnson et al., Methods Cell Biol. 35:73-99 (1991). Hybridization with the labeled probe is carried out using a vast excess of human cot-1 DNA for specific hybridization to the corresponding genomic locus.

Chromosomes are counterstained with 4,6-diamino-2-phenylidole and propidium iodide, producing a combination of C- and R-bands. Aligned images for precise mapping are obtained using a triple-band filter set (Chroma Technology, Brattleboro, VT) in combination with a cooled charge-coupled device camera (Photometrics, Tucson, AZ) and variable excitation wavelength filters. (Johnson et al., Genet. Anal. Tech. Appl., 8:75 (1991).) Image collection, analysis and chromosomal fractional length measurements are performed using the ISee Graphical Program System. (Inovision Corporation, Durham, NC.) Chromosome alterations of the genomic region hybridized by the probe are identified as insertions, deletions, and translocations. These alterations are used as a diagnostic marker for an associated disease.

Example 12: Method of Detecting Abnormal Levels of a Polypeptide in a Biological Sample

A polypeptide of the present invention can be detected in a biological sample, and if an increased or decreased level of the polypeptide is detected, this polypeptide is a marker for a particular phenotype. Methods of detection are numerous, and thus, it is understood that one skilled in the art can modify the following assay to fit their particular needs.

For example, antibody-sandwich ELISAs are used to detect polypeptides in a sample, preferably a biological sample. Wells of a microtiter plate are coated with specific antibodies, at a final concentration of 0.2 to 10 ug/ml. The antibodies are either monoclonal or polyclonal and are produced by the method described in Example 10. The wells are blocked so that non-specific binding of the polypeptide to the well is reduced.

The coated wells are then incubated for > 2 hours at RT with a sample containing the polypeptide. Preferably, serial dilutions of the sample should be used to validate results. The plates are then washed three times with deionized or distilled water to remove unbounded polypeptide.

Next, 50 ul of specific antibody-alkaline phosphatase conjugate, at a concentration of 25-400 ng, is added and incubated for 2 hours at room temperature. The plates are again washed three times with deionized or distilled water to remove unbounded conjugate.

Add 75 ul of 4-methylumbelliferyl phosphate (MUP) or p-nitrophenyl phosphate (NPP) substrate solution to each well and incubate 1 hour at room temperature. Measure the reaction by a microtiter plate reader. Prepare a standard curve, using serial dilutions of a control sample, and plot polypeptide concentration on the X-axis (log scale) and fluorescence or absorbance of the Y-axis (linear scale). Interpolate the concentration of the polypeptide in the sample using the standard curve.

Example 13: Formulation

The invention also provides methods of treatment and/or prevention of

diseases or disorders (such as. for example. any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of a Therapeutic. By therapeutic is meant a polynucleotides or polypeptides of the invention (including fragments and variants), agonists or antagonists thereof, and/or antibodies thereto. in combination with a pharmaceutically acceptable carrier type (e.g., a sterile carrier).

The Therapeutic will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the Therapeutic alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the Therapeutic administered parenterally per dose will be in the range of about 1ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the Therapeutic is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Therapeutics can be are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and

intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics are administered orally, rectally, parenterally, intracistemally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), bucally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Therapeutics of the invention are also suitably administered by sustained-release systems. Suitable examples of sustained-release Therapeutics include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or mirocapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release Therapeutics also include liposomally entrapped Therapeutics of the invention (*see* generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the Therapeutic are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos.

4,485.045 and 4,544,545: and EP 102.324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the Therapeutics of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration. in one embodiment, the Therapeutic is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the Therapeutic uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum

albumin, gelatin, or immunoglobulins: hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The Therapeutic is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Therapeutics generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Therapeutics ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous Therapeutic solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized Therapeutic using bacteriostatic Water-for-Injection.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the Therapeutics of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the Therapeutics may be employed in conjunction with other therapeutic compounds.

The Therapeutics of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the

Therapeutics of the invention include. but are not limited to. alum. alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG, and MPL. In a specific embodiment, Therapeutics of the invention are administered in combination with alum. In another specific embodiment, Therapeutics of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the Therapeutics of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the Therapeutics of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles. mumps, rubella), polio, varicella, tetanus/diptheria, hepatitis A, hepatitis B, haemophilus influenzae B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

The Therapeutics of the invention may be administered alone or in combination with other therapeutic agents. Therapeutic agents that may be administered in combination with the Therapeutics of the invention, include but not limited to, other members of the TNF family, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, cytokines and/or growth factors. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through

separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the Therapeutics of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the Therapeutics of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), TR6 (International Publication No. WO 98/30694), OPG, and neutrokine-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TR6 (International Publication No. WO 98/30694), TR7 (International Publication No. WO 98/41629), TRANK, TR9 (International Publication No. WO 98/56892),TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In certain embodiments, Therapeutics of the invention are administered in combination with antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors. Nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™ (lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). Non-nucleoside reverse transcriptase inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are

not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with Therapeutics of the invention to treat AIDS and/or to prevent or treat HIV infection.

In other embodiments, Therapeutics of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the Therapeutics of the invention, include, but are not limited to, TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™, PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, Therapeutics of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis carinii* pneumonia infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, Therapeutics of the invention are used in any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, Therapeutics

of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an opportunistic cytomegalovirus infection. In another specific embodiment, Therapeutics of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, Therapeutics of the invention are used in any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another specific embodiment, Therapeutics of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, Therapeutics of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the Therapeutics of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.

In a further embodiment, the Therapeutics of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the Therapeutics of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamthoxazole, and vancomycin.

Conventional nonspecific immunosuppressive agents, that may be administered in combination with the Therapeutics of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide

methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells.

In specific embodiments, Therapeutics of the invention are administered in combination with immunosuppressants. Immunosuppressants preparations that may be administered with the Therapeutics of the invention include, but are not limited to, ORTHOCLONE™ (OKT3), SANDIMMUNE™/NEORAL™/SANGDYA™ (cyclosporin), PROGRAF™ (tacrolimus), CELLCEPT™ (mycophenolate), Azathioprine, glucorticosteroids, and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, Therapeutics of the invention are administered alone or in combination with one or more intravenous immune globulin preparations. Intravenous immune globulin preparations that may be administered with the Therapeutics of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, and GAMIMUNE™. In a specific embodiment, Therapeutics of the invention are administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In an additional embodiment, the Therapeutics of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the Therapeutics of the invention include, but are not limited to, glucocorticoids and the nonsteroidal anti-inflammatories, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

In another embodiment, compostions of the invention are administered in

combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the Therapeutics of the invention include, but are not limited to, antibiotic derivatives (e.g., doxorubicin, bleomycin, daunorubicin, and dactinomycin); antiestrogens (e.g., tamoxifen); antimetabolites (e.g., fluorouracil, 5-FU, methotrexate, floxuridine, interferon alpha-2b, glutamic acid, plicamycin, mercaptopurine, and 6-thioguanine); cytotoxic agents (e.g., carmustine, BCNU, lomustine, CCNU, cytosine arabinoside, cyclophosphamide, estramustine, hydroxyurea, procarbazine, mitomycin, busulfan, cis-platin, and vincristine sulfate); hormones (e.g., medroxyprogesterone, estramustine phosphate sodium, ethinyl estradiol, estradiol, megestrol acetate, methyltestosterone, diethylstilbestrol diphosphate, chlorotrianisene, and testolactone); nitrogen mustard derivatives (e.g., mephalen, chorambucil, mechlorethamine (nitrogen mustard) and thiotepa); steroids and combinations (e.g., bethamethasone sodium phosphate); and others (e.g., dicarbazine, asparaginase, mitotane, vincristine sulfate, vinblastine sulfate, and etoposide).

In a specific embodiment, Therapeutics of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or any combination of the components of CHOP. In another embodiment, Therapeutics of the invention are administered in combination with Rituximab. In a further embodiment, Therapeutics of the invention are administered with Rituxmab and CHOP, or Rituxmab and any combination of the components of CHOP.

In an additional embodiment, the Therapeutics of the invention are administered in combination with cytokines. Cytokines that may be administered with the Therapeutics of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, Therapeutics of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In an additional embodiment, the Therapeutics of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the Therapeutics of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A (PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Gorwth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are incorporated herein by reference herein.

In an additional embodiment, the Therapeutics of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the Therapeutics of the invention include, but are not limited to, LEUKINE™ (SARGRAMOSTIM™) and NEUPOGEN™ (FILGRASTIM™).

In an additional embodiment, the Therapeutics of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the Therapeutics of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8,

FGF-9, FGF-10, FGF-11, FGF-12. FGF-13, FGF-14, and FGF-15.

In additional embodiments. the Therapeutics of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Example 14: Method of Treating Decreased Levels of the Polypeptide

The present invention relates to a method for treating an individual in need of an increased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an agonist of the invention (including polypeptides of the invention). Moreover, it will be appreciated that conditions caused by a decrease in the standard or normal expression level of a polypeptide of the present invention in an individual can be treated by administering the agonist or antagonist of the present invention. Thus, the invention also provides a method of treatment of an individual in need of an increased level of the polypeptide comprising administering to such an individual a Therapeutic comprising an amount of the agonist or antagonist to increase the activity level of the polypeptide in such an individual.

For example, a patient with decreased levels of a polypeptide receives a daily dose 0.1-100 ug/kg of the agonist or antagonist for six consecutive days. The exact details of the dosing scheme, based on administration and formulation, are provided in Example 13.

Example 15: Method of Treating Increased Levels of the Polypeptide

The present invention also relates to a method of treating an individual in need of a decreased level of a polypeptide of the invention in the body comprising administering to such an individual a composition comprising a therapeutically effective amount of an antagonist of the invention (including polypeptides and antibodies of the invention).

In one example, antisense technology is used to inhibit production of a polypeptide of the present invention. This technology is one example of a method of decreasing levels of a polypeptide, due to a variety of etiologies, such as cancer. For example, a patient diagnosed with abnormally increased levels of a polypeptide is administered intravenously antisense polynucleotides at 0.5, 1.0, 1.5, 2.0 and 3.0 mg/kg day for 21 days. This treatment is repeated after a 7-day rest period if the treatment was well tolerated. The formulation of the antisense polynucleotide is provided in Example 13.

Example 16: Method of Treatment Using Gene Therapy-Ex Vivo

One method of gene therapy transplants fibroblasts, which are capable of expressing a polypeptide, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention can be amplified using PCR primers which correspond to the 5' and 3' end sequences respectively as set

forth in Example 1 using primers and having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

Example 17: Gene Therapy Using Endogenous Genes Corresponding To

Polynucleotides of the Invention

Another method of gene therapy according to the present invention involves operably associating the endogenous polynucleotide sequence of the invention with a promoter via homologous recombination as described, for example, in U.S. Patent NO: 5,641,670, issued June 24, 1997; International Publication NO: WO 96/29411, published September 26, 1996; International Publication NO: WO 94/12650, published August 4, 1994; Koller et al., *Proc. Natl. Acad. Sci. USA,* 86:8932-8935 (1989); and Zijlstra et al., *Nature,* 342:435-438 (1989). This method involves the activation of a gene which is present in the target cells, but which is not expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made which contain a promoter and targeting sequences, which are homologous to the 5' non-coding sequence of endogenous polynucleotide sequence, flanking the promoter. The targeting sequence will be sufficiently near the 5' end of the polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination. The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter.

The amplified promoter and the amplified targeting sequences are digested with the appropriate restriction enzymes and subsequently treated with calf intestinal phosphatase. The digested promoter and digested targeting sequences are added together in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The construct is size fractionated on an agarose gel then purified by phenol extraction and ethanol precipitation.

In this Example, the polynucleotide constructs are administered as naked

polynucleotides via electroporation. However, the polynucleotide constructs may also be administered with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, precipitating agents, etc. Such methods of delivery are known in the art.

Once the cells are transfected, homologous recombination will take place which results in the promoter being operably linked to the endogenous polynucleotide sequence. This results in the expression of polynucleotide corresponding to the polynucleotide in the cell. Expression may be detected by immunological staining, or any other method known in the art.

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in DMEM + 10% fetal calf serum. Exponentially growing or early stationary phase fibroblasts are trypsinized and rinsed from the plastic surface with nutrient medium. An aliquot of the cell suspension is removed for counting, and the remaining cells are subjected to centrifugation. The supernatant is aspirated and the pellet is resuspended in 5 ml of electroporation buffer (20 mM HEPES pH 7.3, 137 mM NaCl, 5 mM KCl, 0.7 mM Na_2 HPO_4, 6 mM dextrose). The cells are recentrifuged, the supernatant aspirated, and the cells resuspended in electroporation buffer containing 1 mg/ml acetylated bovine serum albumin. The final cell suspension contains approximately $3X10^6$ cells/ml. Electroporation should be performed immediately following resuspension.

Plasmid DNA is prepared according to standard techniques. For example, to construct a plasmid for targeting to the locus corresponding to the polynucleotide of the invention, plasmid pUC18 (MBI Fermentas, Amherst, NY) is digested with HindIII. The CMV promoter is amplified by PCR with an XbaI site on the 5' end and a BamHI site on the 3'end. Two non-coding sequences are amplified via PCR: one non-coding sequence (fragment 1) is amplified with a HindIII site at the 5' end and an Xba site at the 3'end; the other non-coding sequence (fragment 2) is amplified with a BamHI site at the 5'end and a HindIII site at the 3'end. The CMV promoter and the fragments (1 and 2) are digested with the appropriate enzymes (CMV promoter - XbaI and BamHI; fragment 1 - XbaI; fragment 2 - BamHI) and ligated together. The

resulting ligation product is digested with HindIII, and ligated with the HindIII-digested pUC18 plasmid.

Plasmid DNA is added to a sterile cuvette with a 0.4 cm electrode gap (Bio-Rad). The final DNA concentration is generally at least 120 μg/ml. 0.5 ml of the cell suspension (containing approximately $1.5.X10^6$ cells) is then added to the cuvette, and the cell suspension and DNA solutions are gently mixed. Electroporation is performed with a Gene-Pulser apparatus (Bio-Rad). Capacitance and voltage are set at 960 μF and 250-300 V, respectively. As voltage increases, cell survival decreases, but the percentage of surviving cells that stably incorporate the introduced DNA into their genome increases dramatically. Given these parameters, a pulse time of approximately 14-20 mSec should be observed.

Electroporated cells are maintained at room temperature for approximately 5 min, and the contents of the cuvette are then gently removed with a sterile transfer pipette. The cells are added directly to 10 ml of prewarmed nutrient media (DMEM with 15% calf serum) in a 10 cm dish and incubated at 37 degree C. The following day, the media is aspirated and replaced with 10 ml of fresh media and incubated for a further 16-24 hours.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product. The fibroblasts can then be introduced into a patient as described above.

Example 18: Method of Treatment Using Gene Therapy - In Vivo

Another aspect of the present invention is using *in vivo* gene therapy methods to treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences into an animal to increase or decrease the expression of the polypeptide. The polynucleotide of the present invention may be operatively linked to a promoter or any other genetic elements necessary for the expression of the polypeptide by the

target tissue. Such gene therapy and delivery techniques and methods are known in the art. see. for example, WO90/11092. WO98/11779; U.S. Patent NO. 5693622, 5705151. 5580859; Tabata et al., Cardiovasc. Res. 35(3):470-479 (1997); Chao et al., Pharmacol. Res. 35(6):517-522 (1997); Wolff, Neuromuscul. Disord. 7(5):314-318 (1997): Schwartz et al., Gene Ther. 3(5):405-411 (1996); Tsurumi et al., Circulation 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, the polynucleotides of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) Ann. NY Acad. Sci. 772:126-139 and Abdallah B. et al. (1995) Biol. Cell 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapies techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder,

stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard

recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be use to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

Example 19: Transgenic Animals

The polypeptides of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, *e.g.*, baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express polypeptides of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the transgene (i.e., polynucleotides of the invention) into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al.. Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993); introducing nucleic acid constructs into embryonic pleuripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When

it is desired that the polynucleotide transgene be integrated into the chromosomal site of the endogenous gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the transgene has taken place. The level of mRNA expression of the transgene in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of transgenic gene-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the transgene product.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to

produce compound heterozygous or homozygous lines: and breeding to place the transgene on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 20: Knock-Out Animals

Endogenous gene expression can also be reduced by inactivating or "knocking out" the gene and/or its promoter using targeted homologous recombination. (*E.g.*, see Smithies et al., Nature 317:230-234 (1985); Thomas & Capecchi, Cell 51:503-512 (1987); Thompson et al., Cell 5:313-321 (1989); each of which is incorporated by reference herein in its entirety). For example, a mutant, non-functional polynucleotide of the invention (or a completely unrelated DNA sequence) flanked by DNA homologous to the endogenous polynucleotide sequence (either the coding regions or regulatory regions of the gene) can be used, with or without a selectable marker and/or a negative selectable marker, to transfect cells that express polypeptides of the invention *in vivo*. In another embodiment, techniques known in the art are used to generate knockouts in cells that contain, but do not express the gene of interest. Insertion of the DNA construct, via targeted homologous recombination, results in inactivation of the targeted gene. Such approaches are particularly suited in research and agricultural fields where modifications to embryonic stem cells can be used to generate animal offspring with an inactive targeted gene (*e.g.*, see Thomas & Capecchi 1987 and Thompson 1989, *supra*). However this approach can be routinely adapted for use in humans provided the recombinant DNA constructs are directly administered or targeted to the required site *in vivo* using appropriate viral vectors that will be apparent to those of skill in the art.

In further embodiments of the invention, cells that are genetically engineered to express the polypeptides of the invention, or alternatively, that are genetically engineered not to express the polypeptides of the invention (e.g., knockouts) are administered to a patient *in vivo*. Such cells may be obtained from the patient (i.e., animal, including human) or an MHC compatible donor and can include, but are not limited to fibroblasts, bone marrow cells, blood cells (e.g., lymphocytes), adipocytes, muscle cells, endothelial cells etc. The cells are genetically engineered *in vitro* using recombinant DNA techniques to introduce the coding sequence of polypeptides of the invention into the cells, or alternatively, to disrupt the coding sequence and/or endogenous regulatory sequence associated with the polypeptides of the invention, e.g., by transduction (using viral vectors, and preferably vectors that integrate the transgene into the cell genome) or transfection procedures, including, but not limited to, the use of plasmids, cosmids, YACs, naked DNA, electroporation, liposomes, etc. The coding sequence of the polypeptides of the invention can be placed under the control of a strong constitutive or inducible promoter or promoter/enhancer to achieve expression, and preferably secretion, of the polypeptides of the invention. The engineered cells which express and preferably secrete the polypeptides of the invention can be introduced into the patient systemically, e.g., in the circulation, or intraperitoneally.

Alternatively, the cells can be incorporated into a matrix and implanted in the body, e.g., genetically engineered fibroblasts can be implanted as part of a skin graft; genetically engineered endothelial cells can be implanted as part of a lymphatic or vascular graft. (See, for example, Anderson et al. U.S. Patent No. 5,399,349; and Mulligan & Wilson, U.S. Patent No. 5,460,959 each of which is incorporated by reference herein in its entirety).

When the cells to be administered are non-autologous or non-MHC compatible cells, they can be administered using well known techniques which prevent the development of a host immune response against the introduced cells. For example, the cells may be introduced in an encapsulated form which, while allowing for an exchange of components with the immediate extracellular environment, does

not allow the introduced cells to be recognized by the host immune system.

Transgenic and "knock-out" animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of polypeptides of the present invention, studying conditions and/or disorders associated with aberrant expression, and in screening for compounds effective in ameliorating such conditions and/or disorders.

Example 22: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation

Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

In Vitro Assay- Agonists or antagonists of the invention can be assessed for

its ability to induce activation, proliferation. differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of the agonists or antagonists of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured in the presence of either formalin-fixed Staphylococcus aureus Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added 10^5 B-cells suspended in culture medium (RPMI 1640 containing 10% FBS, 5 X 10^{-5}M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and 10^{-5} dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with 3H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The positive and negative controls are IL2 and medium respectively.

In Vivo Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of agonists or antagonists of the invention, or truncated forms thereof. Mice receive this treatment for 4 consecutive days, at which time they are sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with agonists or antagonists of the invention identify the results of the activity of the agonists or antagonists on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic

disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

Flow cytometric analyses of the spleens from mice treated with agonist or antagonist is used to indicate whether the agonists or antagonists specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

Likewise, a predicted consequence of increased mature B-cell representation in vivo is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are compared between buffer and agonists or antagonists-treated mice.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 23: T Cell Proliferation Assay

A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of ^{3}H-thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100 µl/well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1 µg/ml in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from human peripheral blood and added to quadruplicate wells (5×10^4/well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of agonists or antagonists of the invention (total volume 200 ul). Relevant protein buffer and medium alone are controls. After 48 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100 µl of supernatant is removed and stored –20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100 ul of medium containing 0.5 uCi of ^{3}H-thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of ^{3}H-thymidine used as a measure of

proliferation. Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative controls for the effects of agonists or antagonists of the invention.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 24: Effect of Agonists or Antagonists of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of Monocytes and Monocyte-Derived Human Dendritic Cells

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF-α, causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FCγRII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of agonist or antagonist of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

<u>Effect on the production of cytokines</u>. Cytokines generated by dendritic cells, in

particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Thl helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells (10^6/ml) are treated with increasing concentrations of agonists or antagonists of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e..g, R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increase expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are treated 1-5 days with increasing concentrations of agonists or antagonists of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degreesC. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Agonists or antagonists of the invention can be screened

using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the compound to be tested. Cells are suspended at a concentration of 2 x 10^6/ml in PBS containing PI at a final concentration of 5 μg/ml, and then incubaed at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of $5x10^5$ cells/ml with increasing concentrations of agonists or antagonists of the invention and under the same conditions, but in the absence of agonists or antagonists. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in presence of agonist or antagonist of the invention. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use. Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e. g, R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

<u>Oxidative burst.</u> Purified monocytes are plated in 96-w plate at 2-$1x10^5$ cell/well. Increasing concentrations of agonists or antagonists of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20 μl 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of H_2O_2 produced by the macrophages, a standard curve of a H_2O_2 solution of known molarity is performed for each experiment.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 25: Biological Effects of Agonists or Antagonists of the Invention

<u>Astrocyte and Neuronal Assays</u>.

Agonists or antagonists of the invention, expressed in *Escherichia coli* and purified as described above, can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes. The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation assay, for example, can be used to elucidate an agonist or

antagonist of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA 83*:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an agonist or antagonist of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

Fibroblast and endothelial cell assays.

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE_2 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or agonists or antagonists of the invention with or without IL-1α for 24 hours. The

supernatants are collected and assayed for PGE_2 by EIA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without agonists or antagonists of the invention IL-1α for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or agonists or antagonists of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with agonists or antagonists of the invention.

Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP^+) and released. Subsequently, MPP^+ is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP^+ is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotidamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and

Unsicker. J. Neuroscience, 1990).

Based on the data with FGF-2. agonists or antagonists of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of an agonist or antagonist of the invention is first examined in vitro in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm^2 on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days in vitro and are processed for tyrosine hydroxylase, a specific marker for dopminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if an agonist or antagonist of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the agonist or antagonist may be involved in Parkinson's Disease.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 26: The Effect of Agonists or Antagonists of the Invention on the Growth of

Vascular Endothelial Cells

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at 2-5x10^4 cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnique, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An agonist or antagonist of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the compound of the invention may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cell indicates that the compound of the invention inhibits vascular endothelial cells.

The studies described in this example tested activity of a polypeptide of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), agonists, and/or antagonists of the invention.

Example 27: Rat Corneal Wound Healing Model

This animal model shows the effect of an agonist or antagonist of the invention on neovascularization. The experimental protocol includes:

a) Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

b) Inserting a spatula below the lip of the incision facing the outer corner of the eye.

c) Making a pocket (its base is 1-1.5 mm form the edge of the eye).

d) Positioning a pellet, containing 50ng- 5ug of an agonist or antagonist of the invention, within the pocket.

e) Treatment with an agonist or antagonist of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 28: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

A. Diabetic db+/db+ Mouse Model.

To demonstrate that an agonist or antagonist of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al., J. Surg. Res. 52*:389 (1992); Greenhalgh, D.G. *et al., Am. J. Pathol. 136*:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al. Proc. Natl. Acad. Sci. USA 77*:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al., J. Immunol. 120*:1375 (1978); Debray-Sachs, M. *et al., Clin. Exp. Immunol. 51(1)*:1-7 (1983); Leiter *et al., Am. J. of Pathol. 114*:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al., Exp. Neurol. 83(2)*:221-232 (1984);

Robertson *et al.*, *Diabetes 29(1)*:60-67 (1980); Giacomelli *et al.*, *Lab Invest. 40(4)*:460-473 (1979); Coleman, D.L., *Diabetes 31 (Suppl)*:1-6 (1982)). These homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol. 120*:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol. 136*:1235-1246 (1990)).

Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study. Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med. 172*:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily

measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

An agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing. Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group. Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is $64mm^2$, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an agonist or antagonist of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al., Am. J. Pathol. 136:*1235 (1990)). A calibrated lens micrometer is used by a

blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

B. Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various *in vitro* and *in vivo* systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahl*et al.*, *J. Immunol. 115*: 476-481 (1975); Werb *et al.*, *J. Exp. Med. 147*:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *An. Intern. Med. 37*:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors. 5* : 295-304 (1991); Haynes *et al.*, *J. Clin. Invest. 61:* 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes et al., *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In:* Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish

phenomenon in rats (Beck *et al.*, *Growth Factors*. *5:* 295-304 (1991); Haynes *et al.*, *J. Clin. Invest. 61:* 703-797 (1978); Wahl, "Glucocorticoids and wound healing", *In*: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA 86*: 2229-2233 (1989)).

To demonstrate that an agonist or antagonist of the invention can accelerate the healing process, the effects of multiple topical applications of the agonist or antagonist on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to section A, above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is.dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily

measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The agonist or antagonist of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Four groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is $64mm^2$, the corresponding size of the dermal punch. Calculations are made using the following formula:

[Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the healing process and the morphologic appearance of the repaired skin is improved by treatment with an agonist or antagonist of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of < 0.05 is considered significant.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 29: Lymphadema Animal Model

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an agonist or antagonist of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The

main lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosis and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then and ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

Circumference Measurements: Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people then those 2 readings are averaged. Readings are taken from both control and edematous limbs.

Volumetric Measurements: On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other

is dipping the limb to marked area.

Blood-plasma protein measurements: Blood is drawn, spun. and serum separated prior to surgery and then at conclusion for total protein and Ca2+ comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quillitine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 30: Suppression of TNF alpha-induced adhesion molecule expression by a Agonist or Antagonist of the Invention

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an

inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

The potential of an agonist or antagonist of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO_2. HUVECs are seeded in 96-well plates at concentrations of 1 x 10^4 cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 μl of

diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 μl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 μl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 μl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 μl of pNNP reagent must then be added to each of the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 μl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of agonists or antagonists of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides or polypeptides of the invention (e.g., gene therapy).

Example 31: Production Of Polypeptide of the Invention For High-Throughput Screening Assays

The following protocol produces a supernatant containing polypeptide of the present invention to be tested. This supernatant can then be used in the Screening Assays described in Examples 33-42.

First, dilute Poly-D-Lysine (644 587 Boehringer-Mannheim) stock solution

(1mg/ml in PBS) 1:20 in PBS (w/o calcium or magnesium 17-516F Biowhittaker) for a working solution of 50ug/ml. Add 200 ul of this solution to each well (24 well plates) and incubate at RT for 20 minutes. Be sure to distribute the solution over each well (note: a 12-channel pipetter may be used with tips on every other channel). Aspirate off the Poly-D-Lysine solution and rinse with 1ml PBS (Phosphate Buffered Saline). The PBS should remain in the well until just prior to plating the cells and plates may be poly-lysine coated in advance for up to two weeks.

Plate 293T cells (do not carry cells past P+20) at 2 x 10^5 cells/well in .5ml DMEM(Dulbecco's Modified Eagle Medium)(with 4.5 G/L glucose and L-glutamine (12-604F Biowhittaker))/10% heat inactivated FBS(14-503F Biowhittaker)/1x Penstrep(17-602E Biowhittaker). Let the cells grow overnight.

The next day, mix together in a sterile solution basin: 300 ul Lipofectamine (18324-012 Gibco/BRL) and 5ml Optimem I (31985070 Gibco/BRL)/96-well plate. With a small volume multi-channel pipetter, aliquot approximately 2ug of an expression vector containing a polynucleotide insert, produced by the methods described in Examples 8-10, into an appropriately labeled 96-well round bottom plate. With a multi-channel pipetter, add 50ul of the Lipofectamine/Optimem I mixture to each well. Pipette up and down gently to mix. Incubate at RT 15-45 minutes. After about 20 minutes, use a multi-channel pipetter to add 150ul Optimem I to each well. As a control, one plate of vector DNA lacking an insert should be transfected with each set of transfections.

Preferably, the transfection should be performed by tag-teaming the following tasks. By tag-teaming, hands on time is cut in half, and the cells do not spend too much time on PBS. First, person A aspirates off the media from four 24-well plates of cells, and then person B rinses each well with .5-1ml PBS. Person A then aspirates off PBS rinse, and person B, using a12-channel pipetter with tips on every other channel, adds the 200ul of DNA/Lipofectamine/Optimem I complex to the odd wells first, then to the even wells, to each row on the 24-well plates. Incubate at 37 degree C for 6 hours.

While cells are incubating, prepare appropriate media, either 1%BSA in

DMEM with 1x penstrep, or HGS CHO-5 media (116.6 mg/L of CaCl2 (anhyd); 0.00130 mg/L $CuSO_4$-$5H_2O$; 0.050 mg/L of $Fe(NO_3)_3$-$9H_2O$; 0.417 mg/L of $FeSO_4$-$7H_2O$; 311.80 mg/L of Kcl; 28.64 mg/L of $MgCl_2$; 48.84 mg/L of $MgSO_4$; 6995.50 mg/L of NaCl; 2400.0 mg/L of $NaHCO_3$; 62.50 mg/L of NaH_2PO_4-H_20; 71.02 mg/L of Na_2HPO4; .4320 mg/L of $ZnSO_4$-$7H_2O$; .002 mg/L of Arachidonic Acid ; 1.022 mg/L of Cholesterol; .070 mg/L of DL-alpha-Tocopherol-Acetate; 0.0520 mg/L of Linoleic Acid; 0.010 mg/L of Linolenic Acid; 0.010 mg/L of Myristic Acid; 0.010 mg/L of Oleic Acid; 0.010 mg/L of Palmitric Acid; 0.010 mg/L of Palmitic Acid; 100 mg/L of Pluronic F-68; 0.010 mg/L of Stearic Acid; 2.20 mg/L of Tween 80; 4551 mg/L of D-Glucose; 130.85 mg/ml of L- Alanine; 147.50 mg/ml of L-Arginine-HCL; 7.50 mg/ml of L-Asparagine-H_20; 6.65 mg/ml of L-Aspartic Acid; 29.56 mg/ml of L-Cystine-2HCL-H_20; 31.29 mg/ml of L-Cystine-2HCL; 7.35 mg/ml of L-Glutamic Acid; 365.0 mg/ml of L-Glutamine; 18.75 mg/ml of Glycine; 52.48 mg/ml of L-Histidine-HCL-H_20; 106.97 mg/ml of L-Isoleucine; 111.45 mg/ml of L-Leucine; 163.75 mg/ml of L-Lysine HCL; 32.34 mg/ml of L-Methionine; 68.48 mg/ml of L-Phenylalainine; 40.0 mg/ml of L-Proline; 26.25 mg/ml of L-Serine; 101.05 mg/ml of L-Threonine; 19.22 mg/ml of L-Tryptophan; 91.79 mg/ml of L-Tryrosine-2Na-$2H_20$; and 99.65 mg/ml of L-Valine; 0.0035 mg/L of Biotin; 3.24 mg/L of D-Ca Pantothenate; 11.78 mg/L of Choline Chloride; 4.65 mg/L of Folic Acid; 15.60 mg/L of i-Inositol; 3.02 mg/L of Niacinamide; 3.00 mg/L of Pyridoxal HCL; 0.031 mg/L of Pyridoxine HCL; 0.319 mg/L of Riboflavin; 3.17 mg/L of Thiamine HCL; 0.365 mg/L of Thymidine; 0.680 mg/L of Vitamin B_{12}; 25 mM of HEPES Buffer; 2.39 mg/L of Na Hypoxanthine; 0.105 mg/L of Lipoic Acid; 0.081 mg/L of Sodium Putrescine-2HCL; 55.0 mg/L of Sodium Pyruvate; 0.0067 mg/L of Sodium Selenite; 20uM of Ethanolamine; 0.122 mg/L of Ferric Citrate; 41.70 mg/L of Methyl-B-Cyclodextrin complexed with Linoleic Acid; 33.33 mg/L of Methyl-B-Cyclodextrin complexed with Oleic Acid; 10 mg/L of Methyl-B-Cyclodextrin complexed with Retinal Acetate. Adjust osmolarity to 327 mOsm) with 2mm glutamine and 1x penstrep. (BSA (81-068-3 Bayer) 100gm dissolved in 1L DMEM

for a 10% BSA stock solution). Filter the media and collect 50 ul for endotoxin assay in 15ml polystyrene conical.

The transfection reaction is terminated, preferably by tag-teaming, at the end of the incubation period. Person A aspirates off the transfection media, while person B adds 1.5ml appropriate media to each well. Incubate at 37 degree C for 45 or 72 hours depending on the media used: 1%BSA for 45 hours or CHO-5 for 72 hours.

On day four, using a 300ul multichannel pipetter, aliquot 600ul in one 1ml deep well plate and the remaining supernatant into a 2ml deep well. The supernatants from each well can then be used in the assays described in Examples 33-40.

It is specifically understood that when activity is obtained in any of the assays described below using a supernatant, the activity originates from either the polypeptide of the present invention directly (e.g., as a secreted protein) or by polypeptide of the present invention inducing expression of other proteins, which are then secreted into the supernatant. Thus, the invention further provides a method of identifying the protein in the supernatant characterized by an activity in a particular assay.

Example 32: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at

higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51 (1995).) A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xxx-Trp-Ser (SEQ ID NO:920)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway.

Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway. (See Table below.) Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

Ligand	JAKs tyk2	Jak1	Jak2	Jak3	STATS	GAS(elements) or ISRE
IFN family						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS
(IRF1>Lys6>IFP)						
Il-10	+	?	?	-	1,3	
gp130 family						
IL-6 (Pleiotrohic)	+	+	+	?	1,3	GAS
(IRF1>Lys6>IFP)						
Il-11(Pleiotrohic)	?	+	?	?	1,3	
OnM(Pleiotrohic)	?	+	+	?	1,3	
LIF(Pleiotrohic)	?	+	+	?	1,3	
CNTF(Pleiotrohic)	-/+	+	+	?	1,3	
G-CSF(Pleiotrohic)	?	+	?	?	1,3	
IL-12(Pleiotrohic)	+	-	+	+	1,3	
g-C family						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid)	-	+	-	+	6	GAS (IRF1 = IFP >>Ly6)(IgH)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
gp140 family						
IL-3 (myeloid)	-	-	+	-	5	GAS
(IRF1>IFP>>Ly6)						
IL-5 (myeloid)	-	-	+	-	5	GAS

GM-CSF (myeloid)	-	-	+	-	5	GAS
Growth hormone family						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-CAS>IRF1=IFP>>Ly6)
Receptor Tyrosine Kinases						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 33-34, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCC GAAATGATTTCCCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO:921)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:922)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCCCGAAATCTAGATTTCCCCGAAATGATTTCCCCGAAA TGATTTCCCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG CCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCT CCGCCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCC TCGGCCTCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTA GGCTTTTGCAAAAAGCTT:3' (SEQ ID NO:923)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol

acetyltransferase (CAT), luciferase. alkaline phosphatase. B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 33-34.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing NFK-B and EGR promoter sequences are described in Examples 35 and 36. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, Il-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

Example 33: High-Throughput Screening Assay for T-cell Activity.

The following protocol is used to assess T-cell activity by identifying factors, and determining whether supernate containing a polypeptide of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the

GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below). The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml genticin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

During the incubation period, count cell concentration, spin down the required number of cells (10^7 per transfection), and resuspend in OPTI-MEM to a final concentration of 10^7 cells/ml. Then add 1ml of 1×10^7 cells in OPTI-MEM to T25 flask and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with supernatants containing polypeptide of the present invention or polypeptide of the present invention induced polypeptides as produced by the protocol described in Example 31.

On the day of treatment with the supernatant, the cells should be washed and

resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of supernatants being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

Transfer the cells to a triangular reservoir boat, in order to dispense the cells into a 96 well dish, using a 12 channel pipette. Using a 12 channel pipette, transfer 200 ul of cells into each well (therefore adding 100, 000 cells per well).

After all the plates have been seeded, 50 ul of the supernatants are transferred directly from the 96 well plate containing the supernatants into each well using a 12 channel pipette. In addition, a dose of exogenous interferon gamma (0.1, 1.0, 10 ng) is added to wells H9, H10, and H11 to serve as additional positive controls for the assay.

The 96 well dishes containing Jurkat cells treated with supernatants are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 37. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

Example 34: High-Throughput Screening Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity of polypeptide of the present invention by determining whether polypeptide of the present invention proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using

the GAS/SEAP/Neo construct produced in Example 32. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 32, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest 2x10e^{7} U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM $Na_2HPO_4.7H_2O$, 1 mM $MgCl_2$, and 675 uM $CaCl_2$. Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting $1x10^8$ cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of $5x10^5$ cells/ml. Plate 200 ul cells per well in the 96-well plate (or $1x10^5$ cells/well).

Add 50 ul of the supernatant prepared by the protocol described in Example 31. Incubate at 37 degee C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to the protocol described in Example 37.

Example 35: High-Throughput Screening Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation. a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, activation of cells can be assessed by polypeptide of the present invention.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat phenochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by polypeptide of the present invention can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG -3' (SEQ ID NO: 924)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO: 925)

Using the GAS:SEAP/Neo vector produced in Example 32, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and

allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using the Lipofectamine protocol described in Example 31. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as $5x10^5$ cells/ml.

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to $1x10^5$ cells/well). Add 50 ul supernatant produced by Example 31, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay the supernatant according to Example 37.

Example 36: High-Throughput Screening Assay for T-cell Activity

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide

variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB (Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the supernatants produced in Example 31. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO:926), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGAC TTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO:927)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO:922)

PCR amplification is performed using the SV40 promoter template present in the pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene)

Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTTCC ATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC ATCCCGCCCCTAACTCCGCCCAGTTCCGCCCATTCTCCGCCCCATGGCTGA CTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCCTCGGCCTCTGAGCTA TTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAA GCTT:3' (SEQ ID NO:928)

Next, replace the SV40 minimal promoter element present in the pSEAP2-promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes SalI and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with SalI and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 33. Similarly, the method for assaying supernatants with these stable Jurkat T-cells is also described in Example 33. As a positive control, exogenous TNF alpha (0.1,1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

Example 37: Assay for SEAP Activity

As a reporter molecule for the assays described in Examples 33-36, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x

dilution buffer into Optiplates containing 35 ul of a supernatant. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on luminometer, one should treat 5 plates at each time and start the second set 10 minutes later.

Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# of plates	Rxn buffer diluent (ml)	CSPD (ml)
10	60	3
11	65	3.25
12	70	3.5
13	75	3.75
14	80	4
15	85	4.25
16	90	4.5
17	95	4.75
18	100	5
19	105	5.25
20	110	5.5
21	115	5.75
22	120	6
23	125	6.25

24	130	6.5
25	135	6.75
26	140	7
27	145	7.25
28	150	7.5
29	155	7.75
30	160	8
31	165	8.25
32	170	8.5
33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

Example 38: High-Throughput Screening Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify supernatants which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO_2 incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4 , 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated at 37 degrees C in a CO_2 incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10^6 cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10^6 cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as

fluo-4 . The supernatant is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium. the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second: (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by the a molecule, either polypeptide of the present invention or a molecule induced by polypeptide of the present invention, which has resulted in an increase in the intracellular Ca^{++} concentration.

Example 40: High-Throughput Screening Assay Identifying Tyrosine Kinase Activity

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins. Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether polypeptide of the present invention or a molecule induced by polypeptide of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol

is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford,MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford,MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or 50 ul of the supernatant produced in Example 31, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na3VO4, 2 mM Na4P2O7 and a cocktail of protease inhibitors (# 1836170) obtained from Boeheringer Mannheim (Indianapolis, IN) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at $4^{o}C$. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation. the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4

degree C at 16,000 x g.

Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

Generally, the tyrosine kinase activity of a supernatant is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg_{2+} (5mM ATP/50mM $MgCl_2$), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM $MgCl_2$, 5 mM $MnCl_2$, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initial the reaction by adding 10ul of the control enzyme or the filtered supernatant.

The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mm EDTA and place the reactions on ice.

Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavadin coated 96 well plate to associate with the biotinylated peptide. Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phospotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of

tyrosine kinase activity.

Example 41: High-Throughput Screening Assay Identifying Phosphorylation Activity

As a potential alternative and/or compliment to the assay of protein tyrosine kinase activity described in Example 40, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or 50 ul of the supernatants obtained in Example 31 for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the

Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by polypeptide of the present invention or a molecule induced by polypeptide of the present invention.

Example 42: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation

This assay is based on the ability of human CD34+ to proliferate in the presence of hematopoietic growth factors and evaluates the ability of isolated polypeptides expressed in mammalian cells to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of polypeptides on hematopoietic activity of a wide range of progenitor cells, the assay contains a given polypeptide in the presence or absence of other hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested polypeptide has a stimulatory effect on a hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given polypeptide, or agonists or antagonists thereof, might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in cells that are first subjected to *in vitro* stimulation with SCF+IL+3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-

glutamine (500ml) Quality Biological. Inc.. Gaithersburg, MD Cat# 160-204-101). After several gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to 2.5 x 10^5 cells/ml. During this time, 100 μl of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with a given polypeptide in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10 μl of prepared cytokines, 50 μl of the supernatants prepared in Example 31 (supernatants at 1:2 dilution = 50 μl) and 20 μl of diluted cells are added to the media which is already present in the wells to allow for a final total volume of 100 μl. The plates are then placed in a 37°C/5% CO_2 incubator for five days.

Eighteen hours before the assay is harvested, 0.5 μCi/well of [3H] Thymidine is added in a 10 μl volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60 μl Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film A bar code 15 sticker is affixed to the first plate for counting. The sealed plates is then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given polypeptide to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof. As a nonlimiting example, potential antagonists tested in this assay would be expected to inhibit cell proliferation in the presence of cytokines and/or to increase the inhibition of cell proliferation in the presence of cytokines and a given polypeptide. In contrast, potential agonists tested in this assay would be expected to enhance cell proliferation and/or to decrease the inhibition of cell proliferation in the presence of

cytokines and a given polypeptide.

The ability of a gene to stimulate the proliferation of bone marrow CD34+ cells indicates that polynucleotides and polypeptides corresponding to the gene are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

Example 43: Assay for Extracellular Matrix Enhanced Cell Response (EMECR)

The objective of the Extracellular Matrix Enhanced Cell Response (EMECR) assay is to identify gene products (e.g., isolated polypeptides) that act on the hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells to fn is mediated by the $\alpha_5.\beta_1$ and $\alpha_4.\beta_1$ integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and responsible for stimulating stem cell self-renewal has not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of 0.2 $\mu g/ cm^2$. Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem

cells is to be expected. Gene products of the invention (e.g., including, but not limited to, polynucleotides and polypeptides of the present invention, and supernatants produced in Example 31), are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where test factor supernates represent 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5% CO_2, 7% O_2, and 88% N_2) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACScan.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

If a particular polypeptide of the present invention is found to be a stimulator of hematopoietic progenitors, polynucleotides and polypeptides corresponding to the gene encoding said polypeptide may be useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The gene product may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

Additionally, the polynucleotides and/or polypeptides of the gene of interest and/or agonists and/or antagonists thereof, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, polynucleotides and polypeptides corresponding to the gene of

interest may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, for example, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

Example 44: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation

The polypeptide of interest is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the polypeptide of interest on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNFa stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100 μl culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5 μg/ml hEGF, 5mg/ml insulin, 1μg/ml hFGF, 50mg/ml gentamycin, 50 μg/ml Amphotericin B, 5%FBS. After incubation at 37°C for at least 4-5 hours, culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media. 50mg/ml gentamycin, 50μg/ml Amphotericin B, 0.4% FBS. Incubate at 37°C until day 2.

On day 2, serial dilutions and templates of the polypeptide of interest are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or polypeptides of the present invention and incubate at 37°C/5% CO_2 until day 5.

Transfer 60μl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4°C until Day 6 (for IL6 ELISA). To the remaining 100 μl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10μl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 μl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 μl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker. Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 μl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels. Add 100 μl/well of Enhancement Solution and shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay are tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the polypeptide of the present invention may be involved in dermal fibroblast

proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of polypeptides, polynucleotides, agonists and/or antagonists of the polynucleotide/polypeptide of the present invention which gives a positive result. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, polypeptides of the present invention and polynucleotides of the present invention may be used in wound healing and dermal regeneration, as well as the promotion of vasculargenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, antagonists of polypeptides and polynucleotides of the invention may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; artheroscleric plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uvietis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophiliac joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, antagonists of polypeptides and polynucleotides of the invention may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

Example 45: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100 μl of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 μl volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 μl of 0.1% paraformaldehyde-PBS(with Ca++ and Mg++) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10 μl of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 μg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20 μl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution, refered to herein as the working dilution) are added to

each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer: 1:5,000 (10^0) > $10^{-0.5}$ > 10^{-1} > $10^{-1.5}$. 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNNP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

Example 46: Alamar Blue Endothelial Cells Proliferation Assay

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of the protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37-C overnight. After the overnight incubation of the cells, the growth media is removed and replaced with

GIBCO EC-SFM. The cells are treated with the appropriate dilutions of the protein of interest or control protein sample(s) (prepared in SFM) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form. i.e. stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity. The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

Example 47: Detection of Inhibition of a Mixed Lymphocyte Reaction

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by gene products (e.g., isolated polypeptides). Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by these polypeptides since the peripheral blood mononuclear fraction used in this assay includes T, B and

natural killer lymphocytes, as well as monocytes and dendritic cells.

Polypeptides of interest found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM®, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to 2×10^6 cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to 2×10^5 cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of test materials (50 μl) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final concentration of 1 μg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 μg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO_2, and 1 μC of [^{3}H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or supernatant), which enhances proliferation of lymphocytes.

One skilled in the art could easily modify the exemplified studies to test the activity of polynucleotides (e.g., gene therapy), antibodies, agonists, and/or antagonists and fragments and variants thereof.

It will be clear that the invention may be practiced otherwise than as particularly described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and. therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent applications, journal articles, abstracts, laboratory manuals, books, or other disclosures) in the Background of the Invention, Detailed Description, and Examples is hereby incorporated herein by reference. Further, the hard copy of the sequence listing submitted herewith and the corresponding computer readable form are both incorporated herein by reference in their entireties. Moreover. the hard copy of and the corresponding computer readable form of the Sequence Listing of Serial No. 60/124,270 are also incorporated herein by reference in their entireties.

What Is Claimed Is:

1. An isolated nucleic acid molecule comprising a polynucleotide having a nucleotide sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polynucleotide fragment of SEQ ID NO:X or a polynucleotide fragment of the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(b) a polynucleotide encoding a polypeptide fragment of SEQ ID NO:Y or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(c) a polynucleotide encoding a polypeptide fragment of a polypeptide encoded by SEQ ID NO:X or a polypeptide fragment encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(d) a polynucleotide encoding a polypeptide domain of SEQ ID NO:Y or a polypeptide domain encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(e) a polynucleotide encoding a polypeptide epitope of SEQ ID NO:Y or a polypeptide epitope encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X;

(f) a polynucleotide encoding a polypeptide of SEQ ID NO:Y or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X, having biological activity;

(g) a polynucleotide which is a variant of SEQ ID NO:X;

(h) a polynucleotide which is an allelic variant of SEQ ID NO:X;

(i) a polynucleotide which encodes a species homologue of the SEQ ID NO:Y;

(j) a polynucleotide capable of hybridizing under stringent conditions to any one of the polynucleotides specified in (a)-(i), wherein said polynucleotide does not hybridize under stringent conditions to a nucleic acid molecule having a nucleotide

sequence of only A residues or of only T residues.

2. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding a protein.

3. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises a nucleotide sequence encoding the sequence identified as SEQ ID NO:Y or the polypeptide encoded by the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

4. The isolated nucleic acid molecule of claim 1, wherein the polynucleotide fragment comprises the entire nucleotide sequence of SEQ ID NO:X or the cDNA sequence included in the related cDNA clone, which is hybridizable to SEQ ID NO:X.

5. The isolated nucleic acid molecule of claim 2, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

6. The isolated nucleic acid molecule of claim 3, wherein the nucleotide sequence comprises sequential nucleotide deletions from either the C-terminus or the N-terminus.

7. A recombinant vector comprising the isolated nucleic acid molecule of claim 1.

8. A method of making a recombinant host cell comprising the isolated nucleic acid molecule of claim 1.

9. A recombinant host cell produced by the method of claim 8.

10. The recombinant host cell of claim 9 comprising vector sequences.

11. An isolated polypeptide comprising an amino acid sequence at least 95% identical to a sequence selected from the group consisting of:

(a) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(b) a polypeptide fragment of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone, having biological activity;

(c) a polypeptide domain of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(d) a polypeptide epitope of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(e) a full length protein of SEQ ID NO:Y or of the sequence encoded by the cDNA included in the related cDNA clone;

(f) a variant of SEQ ID NO:Y;

(g) an allelic variant of SEQ ID NO:Y; or

(h) a species homologue of the SEQ ID NO:Y.

12. The isolated polypeptide of claim 11, wherein the full length protein comprises sequential amino acid deletions from either the C-terminus or the N-terminus.

13. An isolated antibody that binds specifically to the isolated polypeptide of claim 11.

14. A recombinant host cell that expresses the isolated polypeptide of claim 11.

15. A method of making an isolated polypeptide comprising:

(a) culturing the recombinant host cell of claim 14 under conditions such that said polypeptide is expressed: and

(b) recovering said polypeptide.

16. The polypeptide produced by claim 15.

17. A method for preventing, treating, or ameliorating a medical condition, comprising administering to a mammalian subject a therapeutically effective amount of the polypeptide of claim 11 or the polynucleotide of claim 1.

18. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or absence of a mutation in the polynucleotide of claim 1; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or absence of said mutation.

19. A method of diagnosing a pathological condition or a susceptibility to a pathological condition in a subject comprising:

(a) determining the presence or amount of expression of the polypeptide of claim 11 in a biological sample; and

(b) diagnosing a pathological condition or a susceptibility to a pathological condition based on the presence or amount of expression of the polypeptide.

20. A method for identifying a binding partner to the polypeptide of claim 11 comprising:

(a) contacting the polypeptide of claim 11 with a binding partner; and

(b) determining whether the binding partner effects an activity of the polypeptide.

21. The gene corresponding to the cDNA sequence of SEQ ID NO:Y.

22. A method of identifying an activity in a biological assay, wherein the method comprises:

(a) expressing SEQ ID NO:X in a cell;

(b) isolating the supernatant;

(c) detecting an activity in a biological assay; and

(d) identifying the protein in the supernatant having the activity.

23. The product produced by the method of claim 20.

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